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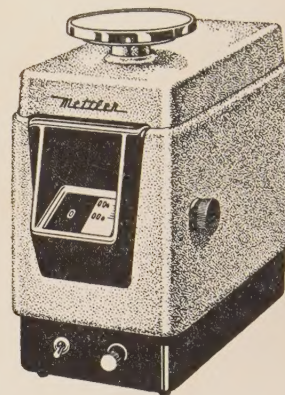
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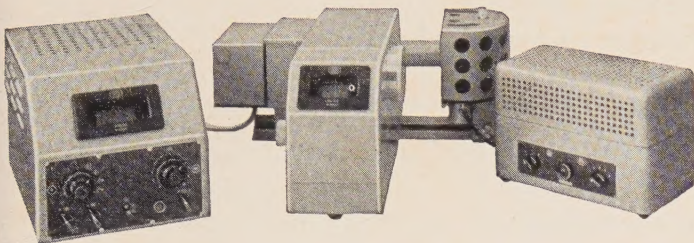
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Nirgends wird die Macht und die innere Kohärenz begrifflichen Denkens so schonungslos auf die Probe gestellt wie in der Mathematik. Im mathematischen Grundlagenproblem wird, wie dieses Werk in eingehender Diskussion zeigt, eine echte Problematik menschlichen Denkens offenbar. Die Untersuchung derselben führt zu wesentlichen Einsichten in die Natur und die Grenzen menschlicher Erkenntnis und damit über den Menschen schlechthin.



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Die Verletzung von Spiegelungs-Symmetrien in den Gesetzen der Atomphysik

Von W. PAULI, ETH, Zürich

I. Kategorien der Wechselwirkung Die Symmetrien C , P und T

Zur Diskussion der Symmetriegrade physikalischer Gesetze ist es zweckmässig, die Wechselwirkungen der Physik in drei Kategorien einzuteilen: die starken Wechselwirkungen, unter welche diejenigen zwischen Nukleonen und zwischen Nukleonen und Mesonen fallen, die mittelstarken elektromagnetischen Wechselwirkungen, die auch für die äussere Atomhülle verantwortlich sind, und die schwachen Wechselwirkungen, zu denen alle Erscheinungen der Betaradioaktivität gehören, die mit Emission oder Absorption von Neutrinos verbunden sind, sowie auch der Zerfall der Λ - und K -Mesonen, in welchem Neutrinos nicht vorkommen.

Die starken Wechselwirkungen haben einen noch höheren Symmetriegrad als die elektromagnetischen, doch ist es für den Zweck dieser Übersicht nicht nötig, hierauf näher einzugehen. Hierfür genügt es, darauf hinzuweisen, dass diese ersten beiden Kategorien, wie aus dem empirischen Material mit grosser Genauigkeit hervorgeht, gegenüber den folgenden drei Symmetrie-Operationen *einzelnen invariant* sind. Jede dieser Operationen ordnet einen möglichen physikalischen Zustand oder Vorgang je einem andern zu, der ebenfalls mit den hierbei betrachteten Naturgesetzen verträglich ist.

1. *Die Teilchen-Antiteilchen-Konjugation (Ladungssymmetrie) C* ¹. Es ist ein allgemeiner Zug der die verschiedenen «Elementarteilchen» und ihre Wechselwirkung regulierenden Naturgesetze, dass zu jedem Teilchen ein Antiteilchen gehört. Sind diese elektrisch geladen, so hat diese Ladung für Teilchen und Antiteilchen entgegengesetztes Vorzeichen. Umgekehrt braucht nicht jedes ähnliche Teilchen entgegengesetzter Ladung wirklich das Antiteilchen zu sein. Und auch zu neutralen Teilchen kann es von ihm verschiedene Antiteilchen geben. So hat das nun experimentell ebenfalls nachgewiesene Antineutron bei gleicher Spinrichtung das entgegengesetzt gerichtete magnetische Moment wie das Neutron. Ob das dem Neutrino entsprechende Antineutrino von diesem experimentell unterscheidbar ist mit Hilfe eines Erhaltungssatzes für die Differenz

der Gesamtzahl der «leichten Teilchen» (Leptonen) minus der Gesamtzahl der «leichten Antiteilchen», ist eine noch offene Frage. Für die entsprechende Differenz von «schweren Teilchen» (Baryonen = Nukleonen und Hyperonen) gilt ein solcher Erhaltungssatz.

2. *Die räumliche Spiegelung oder Paritäts-Symmetrie P* . Diese ändert das Vorzeichen aller drei Raumkoordinaten, ordnet also jeder Rechtsschraube eine Linksschraube zu. Technisch unterscheidet diese räumliche Spiegelungsoperation die sogenannten «axialen Vektoren» von den «polaren Vektoren». Nur die letzteren sind eigentlich durch eine *Länge mit Richtung* charakterisiert, erstere aber durch eine *Fläche mit Umlaufsinn*. Die axialen Vektoren, auch Pseudovektoren genannt, ändern bei Spiegelungen ihr Vorzeichen nicht, die polaren Vektoren kehren es dagegen um. Eine Geschwindigkeit ist ein polarer Vektor, ein Impulsmoment, insbesondere jeder «Spin» ist ein axialer Vektor.

Die Zuordnung einer Richtung zu einem Pseudovektor ist daher nicht spiegel-invariant, sondern in ihrer Definition ist stets eine Linksschraube vor einer Rechtsschraube ausgezeichnet. Drehinvariant ist die Zuordnung der Normale zur Fläche mit Umlaufsinn, die den axialen Vektor darstellt. Welche der beiden Richtungen dieser Normalen aber gewählt werden soll, ist konventionell. Zu deren Festlegung dienen Ampèresche Schwimmerregeln oder äquivalente Vorschriften, wie: sei die Fläche die horizontale Ebene des Papiers, dann weise bei Umlauf im entgegengesetzten Sinne des Uhrzeigers die zugeordnete Normalrichtung auf den Leser zu, nach oben; umgekehrt bei Umlauf im Uhrzeigersinn nach unten. In diesem konventionellen Sinn sind Ausdrücke wie «Spin-Richtung» stets zu verstehen.

Während aus zwei gewöhnlichen (polaren) Vektoren der P -invariante Skalar (Produkt aus den Längen der Vektoren und dem Cosinus ihres Zwischenwinkels) gebildet werden kann, lässt sich aus einem polaren und axialen Vektor nur ein *Pseudoskalar* als Produkt beider definieren, der zwar bei Drehungen des Koordinatensystems invariant ist, bei Raumspiegelung aber das Vorzeichen ändert. Um dieses pseudoskalare Produkt einschliesslich Vorzeichen bilden zu können, muss man nämlich dem axialen Vektor (Fläche mit Umlaufsinn)

¹ Der Buchstabe C bedeutet «charge».

mit Hilfe der eben genannten Konvention eine Richtung der Normalen zuordnen. Das pseudoskalare Produkt ist dann das Produkt der Grösse der Fläche, der Länge des polaren Vektors und des Cosinus des Winkels zwischen dessen Richtung und der konventionellen, (nichtspiegel-invariant) gewählten Normalrichtung zur Fläche des axialen Vektors.

Die Naturgesetze einer räumlich spiegel-invarianten (P -invarianten) Theorie dürfen daher ihre Form nicht ändern; zu jedem Vorgang gibt es in dieser einen andern gleichberechtigten, in dem das Vorzeichen der Pseudoskalare das umgekehrte ist.

Das Wort *Parität* (englisch: parity, französisch: parité) bedeutet bei einer ganzen Zahl deren Unterscheidung nach gerade und ungerade. Die Anwendung dieses Begriffes auf die Raumspiegelungen ergibt sich daraus, dass im Falle der Raumspiegelungs-Invarianz aller Wechselwirkungen gemäss der Wellenmechanik die Eigenzustände in «gerade» und «ungerade» zerfallen, so dass die Wellenfunktionen der geraden Zustände bei der Vorzeichenänderung aller Raumkoordinaten (Spiegelung) unverändert bleiben, während die Wellenfunktionen der ungeraden Zustände hierbei ihr Vorzeichen umkehren. Das so definierte Vorzeichen, $+1$ bei geraden und -1 bei ungeraden Zuständen, heisst die *Parität* des Zustandes.

Für Wechselwirkungen, die nicht P -invariant sind, braucht die Parität der Energiezustände gar nicht zu existieren, indem dann die Wellenfunktionen bei räumlicher Spiegelung kein so einfaches Verhalten mehr aufzuweisen brauchen.

Nach der üblichen Auffassung (Konvention) ändert bei der Operation P die elektrische Ladung ihr Vorzeichen nicht, so dass die elektrische Feldstärke ein polarer, die magnetische Feldstärke ein axialer Vektor ist.

3. *Die Zeitumkehr T* . Diese ist so definiert, dass die Raumkoordinaten, ebenso wie die elektrische Ladung ihr Vorzeichen behalten. Man kann daher diese Operation T präziser als «Umkehr der Bewegungsrichtung» aller Vorgänge definieren. Als Beispiel sei angeführt, dass die Bewegung eines geladenen Massenpunktes in einem äusseren Magnetfeld nur dann T -invariant ist, wenn hierbei die äussere magnetische Feldstärke zugleich ihr Vorzeichen wechselt.

Eine wichtige Konsequenz der T -Invarianz ist das Verschwinden des elektrischen Dipolmomentes der Nukleonen (das analog ist zu dessen Verschwinden bei Molekülen in einem bestimmten Energiezustand ihrer Rotation). Die schwachen Wechselwirkungen sind hierfür übrigens praktisch vernachlässigbar.

Durch neuere experimentelle und theoretische Untersuchungen ist die Frage des Umfanges der Symmetrie der Naturgesetze wieder in den Vordergrund des Interesses gerückt. Es war die mit dem Nobelpreis für Physik von 1957 gekrönte Leistung von LEE und

YANG², nachdrücklich darauf hingewiesen zu haben, dass bei den schwachen Wechselwirkungen (3. Kategorie) die experimentelle Evidenz für die Gültigkeit dieser Symmetrieoperationen völlig ungenügend war. Ferner gaben sie Experimente an, wie gerade bei diesen schwachen Wechselwirkungen diese Symmetrie geprüft werden kann.

Diese und ähnliche Experimente wurden inzwischen vielfach durchgeführt und ergaben endgültig eine Verletzung der C - und der P -Symmetrie bei schwachen Wechselwirkungen. Dies wird im folgenden Abschnitt II noch näher erläutert. Hier sei bereits bemerkt, dass die Frage der Gültigkeit der T -Invarianz bei schwachen Wechselwirkungen experimentell noch unentschieden ist. Theoretisch ist diese äquivalent zur Frage der Gültigkeit der kombinierten Operation CP (oder in umgekehrter Reihenfolge PC). Unter sehr allgemeinen und wohlbegründeten Voraussetzungen, zu denen die für die spezielle Relativitätstheorie charakteristische Lorentz-Invarianz gehört, gilt nämlich das sogenannte CPT -Theorem. Dieses sagt aus, dass aus diesen allgemeinen Voraussetzungen – wir verweisen für Einzelheiten hier auf die Literatur³ – die Invarianz der Theorie für die Zusammensetzung (Produkt) aller drei Operationen C , P und T (in irgend einer Reihenfolge) bereits folgt.

Dieses hat unter anderem zur Folge, dass die Masse von Teilchen und Antiteilchen (allgemeiner die Energiewerte eines Systems von Teilchen und die der zu ihnen C -konjugierten Teilchen) einander gleich sein müssen.

II. Experimentelle Evidenz für Verletzung der Links-Rechts-Symmetrie (P) und der Ladungssymmetrie (C)

Wir können hier nur über die qualitative Seite der Experimente kurz referieren und müssen für die noch im Fluss befindlichen quantitativen Fragen auf die Literatur verweisen.

Das erste, wenn auch keineswegs das einfachste Experiment besteht in der Orientierung der Spins beta-radioaktiver Kerne, wofür eine besondere Technik mit Hilfe von Magnetfeldern bei tiefen Temperaturen bereits entwickelt vorlag. Man untersucht sodann die Frage, ob relativ zur (Spiegel-invarianten) Ebene der Kernspins (konventionell normal zu dieser liegt der Vektor $\vec{I}$) eine Asymmetrie der Emissionsrichtung der Elektronen (Negatonen e_- oder Positonen e_+) vorhanden ist. Mit anderen Worten, man untersucht die Verteilung des Pseudoskalars $(\vec{I} \vec{p}_e) = I p_e \cos \Theta$, worin $\vec{I}$

² T. D. LEE und C. N. YANG, Phys. Rev. 104, 254 (1956).

³ Das CPT -Theorem wurde zuerst von G. LÜDERS, Dan. Mat. Fys. Medd. 28, Nr. 5 (1954), klar erkannt. S. auch Ann. Physics 2, 1 (1957) – Ferner: J. SCHWINGER, Phys. Rev. 82, 914 (1951). – W. PAULI, Niels Bohr and the Development of Physics (Pergamon Press, London 1955), p. 30. – Für nicht lokale Theorien gab R. JOSSE, Helv. phys. Acta 30, 409 (1957), eine dem CPT -Theorem äquivalente Bedingung, die für lokale Theorien identisch erfüllt ist. – Weitere Anwendungen: S. T. D. LEE, R. OEHME und C. N. YANG, Phys. Rev. 106, 340 (1957).

den Vektor des Impulses der emittierten Elektronen und $\Theta = \angle(\vec{I}, \vec{p}_e)$ bedeutet. Diese Verteilung müsste bei Spiegelinvarianz der Theorie symmetrisch zu 0 sein, das heisst es müssten relativ zur Spinebene gleichviel Elektronen in eine Richtung Θ nach vorne und in die Richtung $\pi - \Theta$ nach rückwärts laufen. Die für Co^{60} (e_-) und für Co^{58} (e_+) ausgeführten Experimente⁴ ergaben eine starke Asymmetrie, und zwar im Mittel $(\vec{I} \vec{p}_e) < 0$ für e_- -Zerfall ($\vec{I} \vec{p}_e) > 0$ für e_+ -Zerfall.

Eine nähere Diskussion⁵ zeigt, dass hieraus bereits auch eine Verletzung der C -Symmetrie folgt. In einer C -invarianten Theorie muss dieser Effekt nämlich 0 sein, solange die Coulomb-Wechselwirkung zwischen Kern und emittiertem Elektron vernachlässigt wird. Bei der Kernladungszahl 27 von Co ist überdies der Einfluss der Coulomb-Wechselwirkung noch viel zu klein, um allein den beobachteten Asymmetrie-Effekt erklären zu können.

Ein verwandtes, einfacheres Experiment ist die Messung der Polarisation der beim Betazerfall emittierten Elektronen gegebener Richtung. Die Polarisation ist hierbei bestimmt durch die (im Ruhesystem des Elektrons definierte) Ebene mit Umlaufsinn des Elektronen-Spins (die zugehörige konventionelle Richtung des Vektors $\vec{\sigma}_e$ bildet, vom Teilchen aus gesehen, mit dieser Ebene eine Rechtsschraube), und man misst den Pseudoskalar $(\vec{\sigma}_e \vec{p}_e)$.

Die Experimente haben eine starke Polarisation ergeben⁶, in dem Sinne, dass für e_- -Zerfall im Mittel $(\vec{\sigma}_e \vec{p}_e) < 0$, das heisst axialer Vektor $\vec{\sigma}_e$ und $\vec{p}_e$ bilden eine Linksschraube; für e_+ -Zerfall im Mittel $(\vec{\sigma}_e \vec{p}_e) > 0$, das heisst axialer Vektor $\vec{\sigma}_e$ und $\vec{p}_e$ bilden eine Rechtsschraube.

Von ähnlicher Art ist die Messung des Sinnes der Zirkular-Polarisation von Photonen, deren Emission in einem zweiten Prozess der Emission der Elektronen (Betazerfall in angeregten Kern) nachfolgt. Man misst

hierbei den Pseudoskalar $(\vec{p}_e \vec{\sigma}_\gamma)$. Auch dieses Experiment war positiv⁷.

Wir kommen sodann zu dem wichtigen, ebenfalls auf Veranlassung von LEE und YANG ausgeführten Experiment über den Zerfall des μ -Mesons, das etwa gleichzeitig mit dem ersten Experiment mit gerichteten Kernspins ein positives Resultat ergab⁸.

Man betrachtet π -Mesonen, die gemäss

$$\pi \rightarrow \mu + \nu$$

in μ -Meson und Neutrino zerfallen, worauf dann das μ -Meson weiter in zwei Neutrinos (genauer in ein Neutrino ν und ein Antineutrino $\bar{\nu}$) sowie ein Elektron zerfällt.

$$\mu \rightarrow e + \bar{\nu} + \nu$$

Die erste Reaktion ist der Polarisator des μ -Mesons, die zweite der Analysator. Die beobachtete Unsymmetrie der Elektronenemission, relativ zur Ebene, senkrecht zur Flugrichtung der μ -Mesonen, beweist, dass bei beiden Reaktionen die Spiegelungssymmetrie verletzt ist. Als ein Nebenresultat der starken Polarisation der hierbei entstehenden μ -Mesonen in bezug auf ihre Flugrichtung ergab sich die Möglichkeit einer genauen Messung ihres magnetischen Momentes. Weitere Versuche zur Vorzeichen-Bestimmung des Pseudoskalars $(\vec{\sigma}_\mu \vec{p}_\mu)$ sind im Gange.

Alle hier angeführten Experimente sind im Einklang mit einem *speziellen Modell* für das Neutrino, das von verschiedenen Autoren unabhängig vorgeschlagen wurde⁹. Es wird gewöhnlich etwas ungenau als «Zwei-Komponententheorie» bezeichnet, doch möchte ich es in seinen zwei Varianten kurz « R -Modell» bzw. « L -Modell» nennen. Das « R -Modell» ist dadurch gekennzeichnet, dass nur dasjenige Neutrino, für welches Spin und Bewegungsrichtung eine Rechtsschraube bilden, sowie das zugehörige Antineutrino, bei welchem dann Spin und Impuls notwendig umgekehrt eine Linksschraube bilden, existieren soll. Das bedeutet also, dass beim Neutrino nur für $(\vec{\sigma}_\nu \vec{p}_\nu) > 0$, beim Antineutrino nur für $(\vec{\sigma}_\nu \vec{p}_\nu) < 0$ irgend eine Wechselwirkung mit andern Teilchen stattfindet. Für ein solches Modell sind die C - und die P -Invarianz notwendig verletzt, die CP - und T -Invarianz sind möglich, aber nicht notwendig, und es gilt ein Erhaltungssatz für die Differenz der Teilchen- und Antiteilchenzahlen für die Leptonen. Die bis jetzt vorliegenden Experimente reichen jedoch nicht aus, um die Richtigkeit dieses speziellen Modells

⁴ C. S. WU, E. AMBLER, R. W. HAYWARD, D. D. HOPPEs und R. P. HUDSON, Phys. Rev. 105, 1413 (1957): Co^{60} . – H. POSTMA, W. J. HUISKAMP, A. R. MIEDEMA, M. J. STEENLAND, H. A. TOLHOEK und C. J. GORTER, Physica 23, 259 (1957): Co^{58} . – E. AMBLER, R. W. HAYWARD, D. D. HOPPEs, R. P. HUDSON und C. S. WU, Phys. Rev. 106, 1361 (1957): Co^{60} , Co^{58} . – Analoges Experiment mit polarisierten Neutronen: M. T. BURG, R. J. EPSTEIN, V. E. KROHN, T. B. NOVAY, S. RABOY, G. R. RINGO und V. L. TELEGGI, Phys. Rev. 107, 1731 (1957).

⁵ T. D. LEE und C. N. YANG, Phys. Rev. 104, 254 (1956). – T. D. LEE, R. OEHME und C. N. YANG, Phys. Rev. 106, 340 (1957).

⁶ Zuerst ausgeführt von H. FRAUENFELDER, R. BOBONE, E. VON GOELER, N. LEVINE, H. R. LEWIS, R. N. PEACOCK, A. ROSSI und G. DE PASQUALI, Phys. Rev. 106, 386 (1957): Co^{60} . – Sodann M. GOLDBABER, L. GRODZINS und A. W. SUNYAR, Phys. Rev. 106, 826 (1957): Bremsstrahlung. – S. S. HANNA, R. S. PRESTON, Phys. Rev. 106, 1363 (1957): Cu^{64} . – H. FRAUENFELDER, A. O. HANSON, N. LEVINE, A. ROSSI und G. DE PASQUALI, Phys. Rev. 107, 643 (1957): Möller-Streuung. – M. DEUTSCH, B. GITTELMAN, R. W. BAUER, L. GRODZINS und A. W. SUNYAR, Phys. Rev. 107, 1733 (1957): Ga^{66} , Cl^{34} . – A. DE SHALIT, S. KUPERMAN, H. J. LIPKIN und T. ROTHEN, Phys. Rev. 107, 1459 (1957): Doppelstreuung. – F. BOEHM, T. B. NOVAY, G. A. BARNES und B. STECH, Phys. Rev. (im Druck): N¹³.

⁷ H. SCHOPPER, Phil. Mag. 2, 710 (1957). – H. APPEL und H. SCHOPPER, Z. Phys. 149, 103 (1957). – F. BOEHM und A. H. WAPSTRA, Phys. Rev. 106, 1364 (1957); 107, 1202 und 1462 (1957).

⁸ R. L. GARWIN, L. M. LEDERMAN und M. WEINRICH, Phys. Rev. 105, 1415 (1957): Cyclotron. – J. I. FRIEDMAN und V. L. TELEGGI, Phys. Rev. 105, 1681 (1957): Photoplatten. – S. auch D. BERLEY, T. COFFIN, R. L. GARWIN, L. M. LEDERMAN, M. WEINRICH, Phys. Rev. 106, 835 (1957).

⁹ R. SALAM, Nuovo Cim. 5, 229 (1957). – T. D. LEE, C. N. YANG, Phys. Rev. 105, 1671 (1957). – L. LANDAU, Nuclear Physics 3, 127 (1957).

sicherzustellen. Um in der allgemeinsten Wechselwirkung des Betazerfalls ohne P - und C -Symmetrie die Konstanten festzulegen, sind nämlich weitere Experimente notwendig, die an sich nichts mit Parität zu tun haben und die Richtungskorrelation von Elektron und Neutrino bei der Emission bestimmen. Diese kann man nicht direkt messen, doch ist der Rückstoss der entstehenden schweren Teilchen (der Kerne; beim Zerfall des freien Neutrons: der Protonen) der Messung zugänglich. Aus diesem kann sodann mit Hilfe des Impulssatzes auf die Richtung und Grösse des Neutrinoimpulses zurückgeschlossen werden. Obwohl diese Messungen durch die in den Uranreaktoren zur Verfügung stehenden starken radioaktiven Quellen nunmehr wesentlich erleichtert sind, befinden sie sich immer noch in einem konfuse, einander widersprechenden Zustand, und ihre weitere Entwicklung muss abgewartet werden.

Selbst wenn das spezielle R - bzw. L -Modell für das Neutrino sich bei allen seinen Reaktionen als richtig erweisen sollte, dürfte dies kaum eine genügende Erklärung für die Verletzung der P -Symmetrie bei schwachen Wechselwirkungen im allgemeinen liefern. Denn, wie LEE und YANG bereits in ihrer ersten Arbeit² bemerkt haben, gibt es mit einiger Sicherheit auch paritätsverletzende Wechselwirkungen, in welche das Neutrino gar nicht eingeht. Bei dem sogenannten « Θ - τ -puzzle» führt nämlich die Annahme der P -Invarianz (Erhaltung der Parität bei den betreffenden Reaktionen) zur Notwendigkeit, verschiedene Teilchen mit in guter Näherung gleichen Massen und Lebensdauern anzunehmen¹⁰. Hierfür liess sich keine auch nur einigermaßen plausible Erklärung finden. Bei Annahme von Paritätsverletzung genügt es jedoch, bei diesen verschiedenen Zerfallsreaktionen *ein und dasselbe Teilchen* (K -Meson) anzunehmen, dem dann keine Parität zukommen muss.

III. Ausblick auf theoretische Probleme

Die tieferen ungelösten Schwierigkeiten der quantisierten Feldtheorien, die sich in mathematischen Divergenzen äussern, treten bei den schwachen Wechselwirkungen nicht zutage, da man sich mit den niedrigsten Näherungen der Störungstheorie begnügen kann. Ebensowenig macht sich bei einem rein phänomenologischen Standpunkt der Theorie gegenüber den neuen Phänomenen das Nichtvorhandensein einer theoretischen Herleitung und Deutung der Massen- und Spinwerte der Teilchen in der Natur unmittelbar geltend. Beim jetzigen Stand der Theorie scheint sich daher gegenüber der empirischen Verletzung der C - und P -Symmetrien eher ein Überfluss von formalen Möglichkeiten als eine formale Unerklärbarkeit zu zeigen.

Wenn es sich aber auch diesmal als methodisch richtig erwiesen hat, erst einmal die Frage nach dem *Wie* aufzuwerfen und die tiefere Frage nach dem *Warum* zurückzustellen, so ist diese doch unbeantwortet vorhanden und dürfte in Zukunft noch weiter ihre Berechtigung geltend machen. Ich halte es für verfehlt, sich davon in eine mir oberflächlich scheinende, opportunistisch «Beschwichtigungsphilosophie» (dies Wort stammt von P. EHRENFEST) oder in zu einfache «Patentlösungen» zu flüchten.

Man kann von hier in zwei Richtungen weitergehen. Erstens kann man die Frage, warum die schwachen Wechselwirkungen die C - und P -Symmetrien verletzen, durch die andere Frage ersetzen, warum bei den starken und den elektromagnetischen Wechselwirkungen diese Symmetrien vorhanden sind. Man kann versuchen, diese Symmetrien auf andere speziellere Eigenschaften dieser Wechselwirkungen zurückzuführen.

Zweitens kann man versuchen, einen Zusammenhang der Symmetrieverletzungen im Kleinen mit Eigenschaften des Universums im Grossen aufzufinden und zu begründen¹¹. Dies überschreitet aber die Möglichkeiten der jetzt bekannten Theorien der Gravitation. Die Gravitationswirkungen, die in den anfangs angeführten drei Kategorien der Wechselwirkungen nicht enthalten sind, müssen zwar als noch viel schwächer als die «schwachen Wechselwirkungen» bezeichnet werden. Nach der Gravitationstheorie von EINSTEIN der keine bekannten Phänomene widersprechen, erfüllen die Gravitationswirkungen jedoch alle Symmetrien C , P und T . Auch sind die direkten Einflüsse des Schwerfeldes auf die besprochenen atomaren Phänomene praktisch völlig vernachlässigbar.

Um bei der Frage des Zusammenhanges zwischen dem Kleinen und dem Grossen über vage Spekulationen hinauszugelangen, fehlen daher noch wesentlich neue Ideen. Hiermit soll jedoch nicht die Unmöglichkeit eines solchen Zusammenhanges bestimmt behauptet werden.

So führen uns die hier besprochenen neuen empirischen Ergebnisse in Probleme, deren Lösung vielleicht noch in ferner Zukunft liegt.

Summary

The reflections of charge (C), space-coordinates (P) and time (T) and, particularly in connection with the space reflection, the distinction between polar vector and axial vector, scalar and pseudoscalar products are explained. The three different kinds of strong, medium (electromagnetic) and weak interactions are introduced. While the first two of them fulfil all reflection invariances mentioned separately, LEE and YANG showed (1956) that for the weak interactions no sufficient empirical evidence existed for the reflection invariances, and they also suggested experiments for checking them.

¹⁰ Siehe hierzu R. DALITZ, Phil. Mag. 44, 1068 (1953). – E. FABRI, Nuovo Cim. 11, 479 (1954). – Ferner Proceedings of the sixth Rochester Conference (1956).

¹¹ T. D. LEE, *Weak interaction*, in Proceedings of the seventh Rochester Conference (1957), Abschnitt über *Mach's principle*. – Für die allgemeine Diskussion s. auch E. P. WIGNER, Revs. Modern Phys. 29, 255 (1957).

The qualitative aspect of the experimental results available in November 1957, which show the violation of the *C*- and the *P*-invariance for weak interactions, is reviewed. The methods hereby applied are betadecay of oriented nuclei, polarisation of emitted electrons in beta-decay, beta-gamma-correlation, asymmetry in the decay of μ -mesons generated by π -meson-decay. The solu-

tion of the θ - τ -puzzle by the assumption of a single particle (*K*-meson) without defined parity is mentioned.

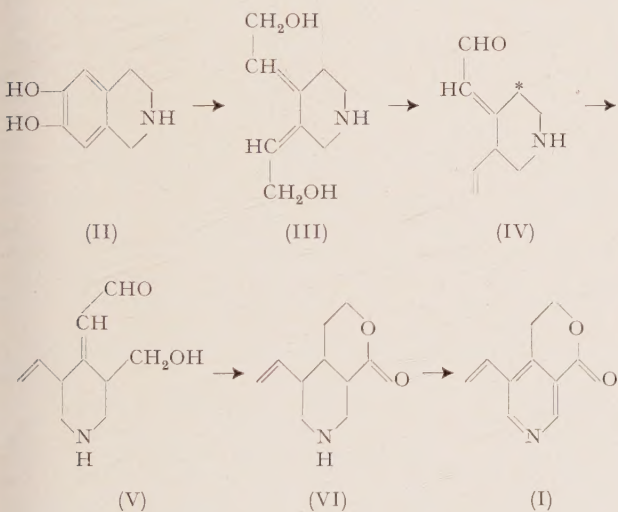
In the concluding section, some aspects of the unsolved theoretical problems of the deeper reasons for the symmetry violations of the weak interactions are briefly discussed which will possibly also lead into open cosmological questions.

Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

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A Biogenetic Scheme for Gentianine

The alkaloid gentianine to which structure (I) has been assigned on the basis of degradation¹ and synthetic evidence², is unique in having carbon substituents at the 3-, 4-, and 5-positions of the pyridine ring. A plausible biosynthetic route to this alkaloid is suggested by the presence of two 2-carbon fragments in adjacent positions. Woodward fission³ of 1:2:3:4-tetrahydro-6:7-dihydroxyisoquinoline (II) would yield the pyridine (III)



which can give by oxidation-reduction and dehydration, the vinyl compound (IV). Attack at the allylic (starred) position in (IV) by formaldehyde or equivalent⁴ would give the alcohol (V), which through oxidation-reduction

to the lactone (VI) and dehydrogenation would yield gentianine (I). The sequence of steps outlined here need not be the same in the plant, but it is of interest that gentianine furnishes the simplest example of an alkaloid whose formation is best explained by invoking a Woodward fission.

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Department of Chemistry, Presidency College, Madras (India), August 13, 1957.

Zusammenfassung

Für die Biogenese des Gentianins wird ein plausibles Reaktionsschema vorgeschlagen.

Testosterone and Methyltestosterone from Hyodesoxycholic Acid

A recent report¹ from these Laboratories described the conversion of hyodesoxycholic acid (3 α ,6 α -dihydroxycholanic acid) (I), the main constituent of hog-bile into the corpus luteum hormone - progesterone. The present communication is a logical extension of these studies to include the preparation of analogous male sex hormones - testosterone and its synthetic homologue, methyltestosterone (VIII).

The diacetate of 3 α ,6 α -dihydroxypregnan-20-one (II), readily obtained¹ from the bile acid (I) in 55% yield by the Meystre-Miescher degradation, was enol-acetylated in carbon tetrachloride solution with acetic anhydride and a trace of perchloric acid (70-72%)². In view of the geometrical isomers, anticipated on the formation of the Δ^{17} -double bond³, no attempt was made to isolate the triacetate (III), which was directly

¹ T. R. GOVINDACHARI, K. NAGARAJAN, and S. RAJAPPA, *J. chem. Soc. 1957*, 551. - N. F. PROSKURNINA, *J. gen. Chem., Moscow*, **14**, 1148 (1944). - N. F. PROSKURNINA, V. V. SHPANOV, and R. A. KONOVALOVA, *Doklady Akad. Nauk SSSR*, **66**, 437 (1949). - N. F. PROSKURNINA and V. V. SHPANOV, *Zh. Obshch. Khim.*, **26**, 936 (1956).

² T. R. GOVINDACHARI, K. NAGARAJAN, and S. RAJAPPA, *J. chem. Soc. 1957*, 2725.

³ R. B. WOODWARD, *Nature*, **162**, 155 (1948).

⁴ A. LAPWORTH, *J. chem. Soc.*, **79**, 1276 (1901). - W. BORSCHKE and R. MANTEUFFEL, *Lieb. Ann.*, **505**, 177 (1933). - R. ROBINSON, *The Structural Relations of Natural Products* (Oxford, 1955), p. 15.

¹ K. R. BHARUCHA, G. C. BUCKLEY, C. K. CROSS, L. J. RUBIN, and P. ZIEGLER, *Can. J. Chem.*, **34**, 982 (1956).

² D. H. R. BARTON, R. M. EVANS, J. C. HAMLET, P. G. JONES, and T. WALKER, *J. chem. Soc. 1954*, 747.

³ C. W. MARSHALL, T. H. KRITCHEVSKY, S. LIEBERMAN, and T. F. GALLAGHER, *J. Amer. chem. Soc.*, **70**, 1837 (1948). - L. F. FIESER and HUANG-MINLON, *J. Amer. chem. Soc.*, **71**, 1840 (1949).

ozonised *in situ* in a mixture (1:1) of carbon tetrachloride and aqueous acetic acid (95% v/v). Reductive decomposition of the ozonide with zinc dust, followed by saponification, gave 3 α ,6 α -dihydroxyaetiocholan-17-one (IVa), m.p. 246–250°, $[\alpha]_D^{25} + 52.65$ (*c*, 1.675⁴) (Found: C, 74.46; H, 9.97; O, 15.63%. C₁₉H₃₀O₃ requires C, 74.47; H, 9.87; O, 15.66%), characterised as its diacetate (IVb), m.p. 141–143°, $[\alpha]_D^{26} + 65.28$ (*c*, 1.034) (Found: C, 70.58; H, 8.70; O, 20.7%. C₂₃H₃₄O₅ requires C, 70.76; H, 8.71; O, 20.51%). The conversion of 3 α ,6 α -dihydroxy groups of (IVa) into 3 β -hydroxy- Δ^5 -moiety, a precursor of 3 keto- Δ^4 -grouping was effected following the method developed earlier in these Laboratories⁵. The ditosylate (IVc), m.p. 158–159° (decomp.), $[\alpha]_D^{27} + 40.03$ (*c*, 1.768) (Found: C, 64.63; 64.62; H, 6.92; 6.95; O, 18.24; S, 10.42%. C₃₃H₄₂O₇S₂ requires C, 64.47; H, 6.89; O, 18.22; S, 10.43%), derived from the diol (IVa) in near quantitative yield, by treatment with pyridine and *p*-toluenesulphonyl chloride, was heated with potassium acetate in aqueous dimethylformamide at 100° for 4½ h. Sa-

ponification and chromatographic purification then afforded the known Δ^5 -dehydroepiandrosterone (V) (34% overall yield from II), m.p. and mixture m.p. 148–151°, convertible to testosterone by well-established methods⁶.

The synthesis of the higher homologue – methyltestosterone (VIII) from (V) was accomplished via Grignard and Oppenauer reactions. Addition of methylmagnesium iodide in ether to the ketone (V) was initially reported by Ruzicka *et al.*⁷, to give 17 α -methyl-3 β ,17 β - Δ^5 -androstenediol (VI), the yield (57%) being subsequently improved⁸ to 74% by the simple expedient of using a ten-fold excess of the Grignard reagent. In the present work, methylmagnesium chloride, readily prepared in 90% yield, in tetrahydrofuran, was utilised and afforded the 17 α -methyl-diols (VI), m.p. and mixture m.p. 199–202°, in *ca.* 80% yield. Similar result was obtained, when dehydroepiandrosterone acetate was substituted

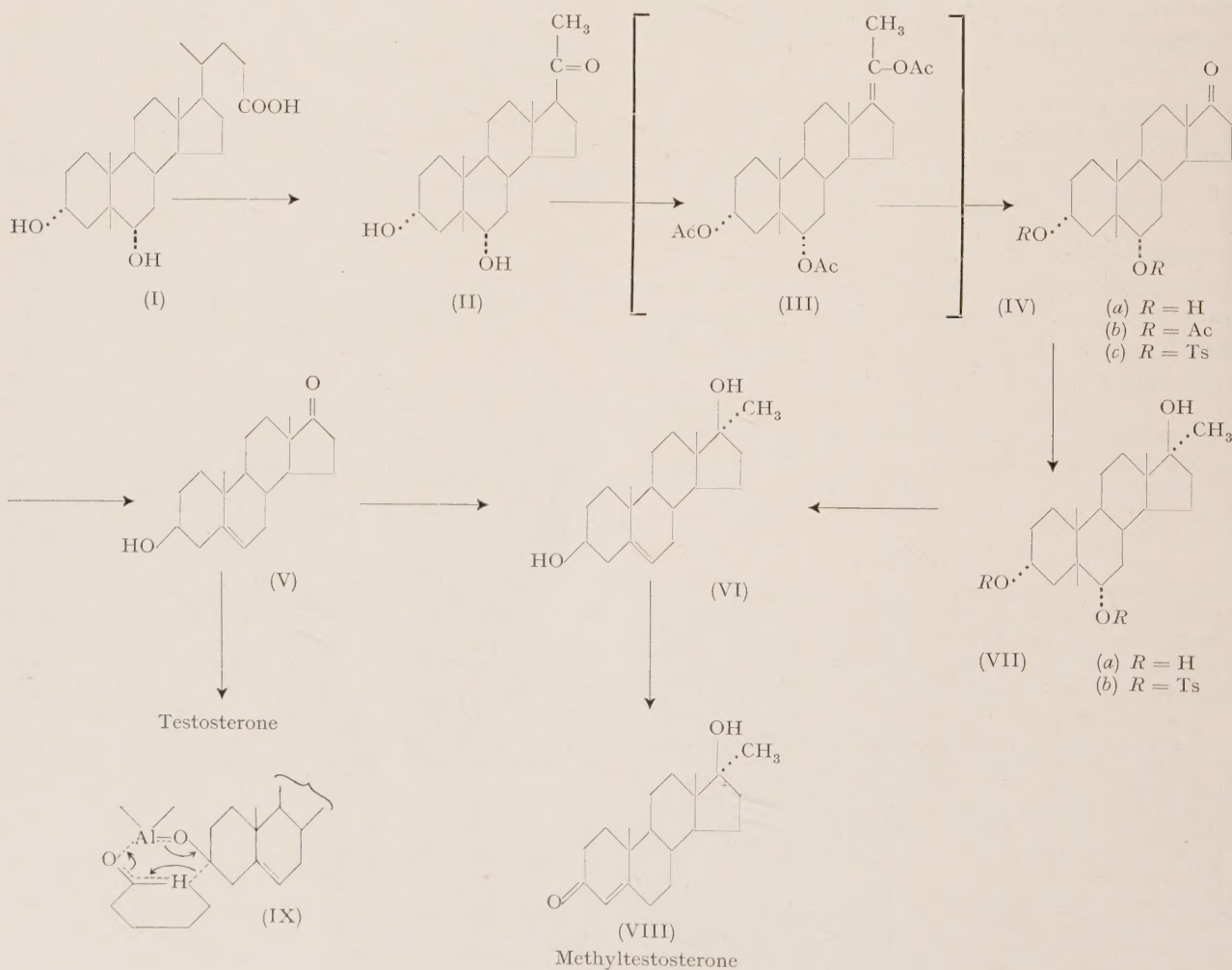
⁴ All rotations were measured in a 1 dm tube, in dioxane, unless otherwise stated.

⁵ K. R. BHARUCHA, G. C. BUCKLEY, C. K. CROSS, L. J. RUBIN, and P. ZIEGLER, *Can. J. Chem.* **34**, 982 (1956). – P. ZIEGLER and K. R. BHARUCHA, *Chem. and Ind.* **1955**, 1351. – See also L. VARGHA and M. RADOS, *Chem. and Ind.* **1955**, 896. – L. VARGHA, M. RADOS, and M. KRAUT, *Acta chim. Acad. Sci. Hung.* **8**, 303 (1955). – S. BERGSTROM and K. PAABO, *Acta chem. Scand.* **9**, 699 (1955).

⁶ *Inter alia*: L. F. FIESER and M. FIESER, *Natural Products Related to Phenanthrene* (Reinhold Publishing Corp., New York 1949), p. 371. – A. C. OTT, M. F. MURRAY, and R. L. PEDERSON, *J. Amer. chem. Soc.* **74**, 1239 (1952). – F. SONDHEIMER, C. AMENDOLLA, and G. ROSENKRANZ, *J. Amer. chem. Soc.* **75**, 5930 (1953). – H. J. DAUBEN, JR., B. LÖKEN, and H. J. RINGOLD, *J. Amer. chem. Soc.* **76**, 1359 (1954).

⁷ L. RUZICKA, M. W. GOLDBERG, and H. R. ROSENBERG, *Helv. chim. Acta* **18**, 1487 (1935).

⁸ G. I. KIPRIANOV and B. E. FRENKEL, *J. gen. Chem., Moscow* **9**, 1682 (1939); *Chem. Abstr.* **34**, 3756 (1940).



for the free hydroxy compound (V). With methyl-lithium in ether, the addition reaction was very rapid (30 min), but the yield (70%) was somewhat diminished. The final step in the projected transformation was completed by Oppenauer oxidation with cyclohexanone and aluminium isopropoxide in the usual manner to furnish methyltestosterone (VIII), m.p. and mixture m.p. 164–166°, $\epsilon_{\max}$ 2410 Å = 16,300 (in EtOH), in 87% yield. An interesting modification was discovered during the course of these investigations and consisted in the use of activated alumina in place of the conventional alkoxide catalyst. Thus, the diol (VI), on treatment with cyclohexanone and alumina (3 g/g steroid) at the reflux temperature of toluene for 1 h was smoothly converted into methyltestosterone (VIII) in 80% yield, taking into consideration a 17% recovery of starting material. The heterogeneous oxidation, though closely related to, is mechanistically different from the Oppenauer reaction, which involves a quasi six-membered cyclic transition state⁹, depicted in (IX). It is unlikely that such an activated complex obtains with the alumina catalysed oxidation, which presumably entails co-adsorption of the diol (VI) and cyclohexanone on the active surface of the catalyst with subsequent hydrogen transfers from the steroid-donor to the ketone-acceptor. In this respect, the reaction is reminiscent, in general, of the heterogeneously catalysed transferhydrogenations, studied extensively by BRAUDE, Linstead *et al.*¹⁰, and in particular, of the Raney nickel catalysed oxidations¹¹ with cyclohexanone.

In an alternative route to the diol (VI) from the dihydroxy-ketone (IVa), the sequence of reactions was reversed, i.e., the C₁₇ side-chain was elaborated preparatory to the construction of the 3 β -hydroxy- Δ^5 -grouping. Treatment of (IVa) with methylmagnesium chloride in tetrahydrofuran under conditions identical to those used with (V), surprisingly gave a comparatively low yield (45%) of 17 α -methyl-3 α , 6 α , 17 β -aetiocholantriol (VIIa), m.p. 230–232°, $[\alpha]_D^{25}$ – 23.47 (*c*, 0.649) (Found: C, 74.27; H, 10.61; O, 15.10%. C₂₀H₃₄O₃ requires C, 74.49; H, 10.63; O, 14.89%). *A priori*, this was ascribed to the poor solubility of (IVa) in the reaction medium, particularly as some (12%) recovery of the starting material was made. However, this could hardly have been the reason, since replacement of (IVa) by its diacetate (IVb), which is quite soluble in tetrahydrofuran and gives a soluble Grignard complex, still failed to improve the yield. Tosylation of the triol (VIIa) with pyridine and tosyl chloride at 0–5°, and subsequent dehydrotosylation of the resultant ditosylate (VIIb), m.p. 145° decomp., $[\alpha]_D^{25}$ – 10.74 (*c*, 0.849) (Found: C, 64.70; H, 7.49; O, 17.98; S, 10.22%. C₃₄H₄₆O₇S₂ requires C, 64.73; H, 7.35; O, 17.75; S, 10.16%), as described above with (IVc), furnished 17 α -methyl-3 β , 17 β - Δ^5 -androstenediol (VI), m.p. and mixture m.p. 197–201°, $[\alpha]_D^{25}$ – 74.2 (*c*, 1.105, alcohol) in 45% yield. The isolation of pure (VI) was a matter of considerable difficulty and was best achieved by conversion to, and regeneration from its oxalic acid adduct¹².

⁹ R. B. Woodward, N. L. Wendler, and F. J. Brutschy, J. Amer. chem. Soc. 67, 1428 (1945). – L. M. Jackman and A. K. Macbeth, J. chem. Soc. 1952, 3252. – W. von E. Doering and T. C. Aschner, J. Amer. chem. Soc. 75, 393 (1953).

¹⁰ E. A. Braude and R. P. Linstead, J. chem. Soc. 1954, 3544, and subsequent papers in this series.

¹¹ E. C. Kleiderer and E. C. Kornfeld, J. org. Chem. 13, 455 (1948).

¹² K. Miescher and H. Kägi, Helv. chim. Acta 24, 986 (1941). – L. Yoder, U.S. Patent, 2,362,605 (1944).

Full details of the work will be published at a later date, elsewhere.

K. R. BHARUCHA

Research Laboratories, Canada Packers Limited, Toronto, July 29, 1957.

Zusammenfassung

Der Hauptbestandteil der Gallenflüssigkeit des Schweines, Hyodesoxycholsäure, wurde in die männlichen Geschlechtshormone Testosteron und Methyltestosteron überführt. Es wird eine modifizierte Oppenauer-Oxydation beschrieben, bei der Aluminiumoxyd an Stelle des gebräuchlichen Alkoxyd-Katalysators verwendet wird.

On the Role of the 4-Formyl Group of the Pyridoxal-5-phosphate in the Activation of Apotransaminase

In a paper by COHEN¹, the inhibition of transaminase activity by cyanide ions was ascribed to their action on the oxalacetic (or pyruvic) acid resulting in cyanohydrin formation. This assumption on the inhibition mechanism was never modified even when pyridoxal-5-phosphate (Py-5-P) was recognized as the coenzyme of transaminase reactions.

In the attempt to demonstrate an alternative inhibition mechanism characterized by cyanohydrin formation due to the reaction between CN-ions and the 4-formyl group of Py-5-P, we have followed the transaminase reaction (a) after addition to the activated system of KCN, and (b) after addition to apotransaminase of Py-5-P previously incubated with KCN.

Our results do not appear to be in agreement with the accepted role played by 4-formyl group of Py-5-P in the scheme of SCHLENK and FISHER², suggesting a particular role of this group in the attachment of the coenzyme to apotransaminase.

The glutamic-oxalacetic transaminase (GOT), used in our experiments, was prepared from pig heart as described by O'KANE and GUNSALUS³, and purified up to the stage of heating at 60°C; the resolution was of about 95% and specific activity of 3.4 (μ M of oxalacetic acid formed by 1 mg protein per minute).

The formation of oxalacetic acid was followed at 280 μ in a Beckman spectrophotometer mod. DU, equipped with thermospacer kept at 37°C. All other conditions of transamination reaction were those proposed by CAMMARATA and COHEN⁴.

The reaction between Py-5-P and KCN was allowed to occur in stoichiometric amounts of Py-5-P (Hoffmann-La Roche) and KCN in phosphate buffer 0.05 M, pH 7.4, for 90 min at 50°C. This addition reaction can be followed spectrophotometrically by the decrease of extinction at 385 μ (Fig. 1). The velocity constant is 4.65 liter mole⁻¹ sec⁻¹ at 37°C. At 50°C the reaction is obviously faster and attains the completeness in 90 min. Full details regarding the kinetics of the above reaction will be reported elsewhere.

¹ P. P. COHEN, Biochem. J. 33, 1478 (1939).

² F. SCHLENK and A. FISHER, Arch. Biochem. 12, 69 (1947).

³ D. E. O'KANE and I. C. GUNSALUS, J. biol. Chem. 170, 425 (1947).

⁴ P. S. CAMMARATA and P. P. COHEN, J. biol. Chem. 193, 53 (1951).

(a) When GOT, previously incubated for 10 min at 37°C with the optimal amount of Py-5-P (Fig. 2), is reincubated with KCN in large excess ($10^{-2} M$), for 25 min at 37°C, in phosphate buffer 0.05 M, pH 7.4, and subsequently dialysed for 12 h against the same buffer, the enzymatic system shows the same activity as that shown in similar experiments without KCN, simultaneously run.

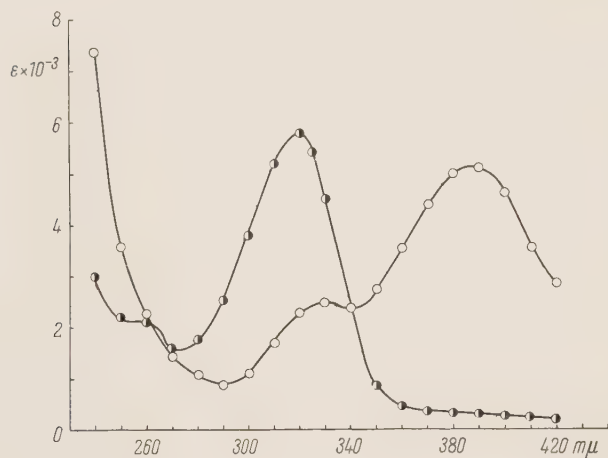


Fig. 1.—Absorption spectra at pH 7.4: o-o, pyridoxal-5-phosphate; ●-●, pyridoxal-5-phosphate after reaction with equivalent KCN.

(b) The Py-5-P, after reaction with KCN in stoichiometric amounts, fails to activate the apotransaminase. Further, its addition even in seven fold excess (either simultaneously or after Py-5-P) to the resolved GOT does not modify the activation effect due to increasing amounts of Py-5-P, at least in our experimental conditions.

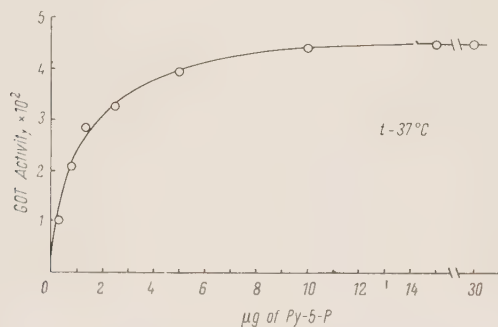


Fig. 2.—GOT activity as a function of pyridoxal-5-phosphate added to the incubation mixture containing 1 ml of the 1:20 enzymesolution. GOT activity $\times 10^2$ equal to one corresponds to 0.034 μ Moles oxaloacetate formed by 1 mg protein per minute.

A role of 4-formyl group quite different from that suggested by SCHLENK and FISHER² may be inferred from our results. It seems, indeed, that the 4-formyl group must be involved in the attachment of the coenzyme to apotransaminase rather than in the formation of a Schiff base with aminoacid substrate.

V. BONAVITA and V. SCARDI

Institute of Human Physiology, University of Naples (Italy), August 1, 1957.

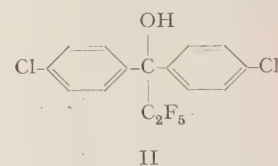
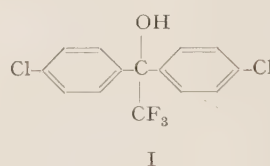
Riassunto

Vengono descritte indagini sull'interazione tra piridossal-5-fosfato e KCN. Il composto di addizione non attiva l'apotransaminasi, ma neppure compete con il piridossal-5-fosfato.

I risultati lasciano intravedere che il gruppo carbonilico di quest'ultimo sia interessato nell'attacco del coenzima stesso all'apoenzima.

Reduced Oviposition in *Aedes aegypti* L. Following Tarsal Exposure to a Fluorocarbon¹

It has been shown recently² that di-(*p*-chlorophenyl)-trifluoromethylcarbinol (I) and di-(*p*-chlorophenyl)-pentafluoroethylcarbinol (II) synthesized by BERGMANN *et al.*³ reduce substantially or even inhibit completely oviposition in the housefly upon tarsal contact.



Although II was pronouncedly better as an 'O.I.T.C.-agent' in houseflies on continuous exposure² than I, in the present work on *Aedes aegypti*, the more readily available I was employed. It was soon found that mosquitoes kept in continuous contact with deposits of I showed even at very low concentrations either an enhanced mortality due to the slight toxicity of the compound⁴, or refused to take their blood meals. Therefore, tarsal contact of short duration followed by a single blood meal was found to be the method of choice.

The number of eggs deposited by *Aedes aegypti* after a single blood meal depends on sufficient larval diet⁵, on fertilization of the females⁶ and on the amount of blood ingested⁷. Accordingly, 4-5 days old females were taken out from mixed population cages⁸ of *Aedes aegypti* bred from well-fed larvae and kept at 28°C, 80% R.H. on 10% glucose. The females were exposed at 24°, under an inverted glass funnel to a deposit of 1 g/sq.m. I on filter paper, exposure times being 10, 20, 30 and 40 min. After the exposure, they were returned to 28°, starved for 20-24 h and then were allowed to feed for 3 h on 'Nembutal'-immobilized guinea pigs. All engorged females

¹ The abbreviation O.I.T.C.-agents (oviposition-inhibiting tarsal contact agents) is suggested for compounds reducing or inhibiting oviposition in insects upon tarsal contact.

² K. R. S. ASCHER, *Science* **125**, 938 (1957).

³ E. D. BERGMANN, A. S. TAHORI, A. KALUSZYNER, and S. REUTER, *Nature* **176**, 266 (1955). — A. KALUSZYNER, S. REUTER, and E. D. BERGMANN, *J. Amer. chem. Soc.* **77**, 4164 (1955).

⁴ S. REUTER and K. R. S. ASCHER, *Exper.* **12**, 316 (1956).

⁵ F. WEYER, *Arch. Schiffs- u. Tropenhyg.* **38**, 394 (1934). — M. MATHIS, *Bull. Soc. Pat. exot.* **36**, 640 (1938). — W. W. MACDONALD, *Ann. trop. Med. Parasit.* **50**, 399 (1956).

⁶ J. D. GILLET, *Nature* **176**, 124 (1955). — J. D. GILLET, *Ann. trop. Med. Parasit.* **50**, 362 (1956). — C. A. LANG, *Amer. J. trop. Med. Hyg.* **5**, 909 (1956). — R. C. WALLIS and C. A. LANG, *Mosquito News* **16**, 283 (1956).

⁷ D. N. ROY, *Bull. ent. Res.* **27**, 423 (1936). — P. A. WOKE, *Amer. J. trop. Med.* **17**, 729 (1937). — P. A. WOKE, M. S. ALLY, and C. S. ROSENBERGER, *Ann. ent. Soc. Amer.* **49**, 435 (1956).

⁸ D. R. SEATON and W. H. R. LUMSDEN, *Ann. trop. Med. Parasit.* **35**, 23 (1941), found that 82% of females in cages had mated 72 h after emergence.

were divided in groups of 10 (Series I) or groups of approximately 25 (Series II) into small cages and equal numbers of males were added. Finally the cages were provided with glucose-solution and an oviposition surface (wet cotton wool covered with a disk of filter paper in a small Petri-dish).

Exposure times up to 40 min caused neither an initial k.d. nor a significant 24 h mortality. Feeding was not inhibited (the mean ingested quantity of blood in treated groups and controls was found to be 2.0 mg), nor was there any difference in mean longevity. Longer exposure than 40 min, however, irritated the mosquitoes and prevented adequate feeding.

Results.—Results are summarized in the Table. Series I is based on 5 × 10 ♀ for each exposure time and for control, while series II was carried out with two groups of approximately 25 ♀ for each experimental arrangement. Females dying within three days after exposure were excluded from the calculations, since oviposition started on the third to fourth day after the blood meal.

	Series I		Series II	
	Combined mean no. of eggs/♀	Mean longevity of females, days	Mean no. of eggs/♀	
			Group 1	Group 2
Control	61.0	12.9	59.6	65.2
10 min	62.3	13.2	58.4	62.8
20 min	47.2	12.2	52.3	48.7
30 min	29.5	12.5	33.8	35.1
40 min	27.2	12.1	30.6	36.0

Repeating these experiments with the ‘one female per cage’-method of Woke *et al.*⁹, it was found that at an exposition time of 30 min only 10 ♀ out of 32 layed eggs (31%), while in controls, 34 ♀ out of 44 (i.e. 77%) deposited eggs.

Unfortunately, rather protracted similar experiments with DDT-resistant strains of *Anopheles stephensi* List. and *Anopheles m. atroparvus* Thiel, using for short-time exposure a magnified form of the BUSVINE-NASH technique¹⁰ followed by two blood meals (one blood meal not insuring oviposition), did not yield reproducible results.

A full account of this work will be published in the *Rendiconti dell’Istituto Superiore di Sanità*.

Acknowledgement. I wish to express my thanks to Dr. E. MOSNA and Dr. S. BETTINI for help, advice and laboratory facilities afforded at the Laboratorio di Parassitologia, Istituto Superiore di Sanità, Roma and to the Direzione Generale delle Relazioni Culturali con l’Estero, Roma, for a research scholarship.

K. R. S. ASCHER

Laboratorio di Parassitologia, Istituto Superiore di Sanità, Roma, August 1, 1957.

Zusammenfassung

Durch Tarsalkontakt während 30–40 min mit Di-(*p*-chlorphenyl)-trifluormethylkarbinol, 20–24 h vor einer einzelnen Blutmahlzeit, konnte der Prozentsatz Eierlegender Gelbfiebermückenweibchen gegenüber der Kontrolle um mehr als die Hälfte reduziert und dadurch die Zahl der abgelegten Eier auf etwa 50% herabgesetzt werden.

⁹ P. A. WOKE, M. S. ALLY, and C. S. ROSENBERGER, *Ann. ent. Soc. Amer.* **49**, 435 (1956).
¹⁰ J. R. BUSVINE and R. NASH, *Bull. ent. Res.* **44**, 371 (1953).

Distribution of DNA and RNA in Different Regions of Cat’s Brain

Nucleic acids play one of the most important roles in cell metabolism (e.g. in synthesis of specific protein-enzymes). Furthermore, recent investigations strongly suggest that nucleoproteins are consumed and regenerated by the nerve cells in response to increased functional demands¹. Since the morphological structure of various parts of the central nervous system (CNS) is different, their metabolic or functional activities may also be supposed to differ significantly. Therefore, the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content of the total brain cannot be considered as an adequate index of the metabolism of these substances in different regions of the CNS.

Region of the brain	No. of animals	DNA	No. of animals	RNA
		γ/10 mg of dried tissue		γ/10 mg of dried tissue
Frontal cortex	8	32.0 ± 2.4	9	299.0 ± 27.6
Occipital cortex	8	31.5 ± 3.1	9	303.4 ± 28.7
Temporal cortex	9	29.3 ± 2.7	9	305.6 ± 11.0
Hippocampus	9	30.7 ± 2.4	8	290.3 ± 27.8
Cerebellar cortex	7	49.2 ± 5.6	8	337.6 ± 23.9
Cerebral white matter . .	6	34.3 ± 4.0	7	148.8 ± 11.4
Caudate nucleus	7	36.7 ± 3.5	9	282.2 ± 19.1
Thalamus	7	34.2 ± 3.6	9	232.4 ± 22.7
Hypothalamus	6	41.8 ± 3.3	5	265.0 ± 23.1
Midbrain	6	32.5 ± 3.8	9	206.1 ± 15.1
Pons	8	31.0 ± 3.0	9	168.0 ± 19.3
Medulla	9	31.2 ± 3.3	7	170.1 ± 7.4
Spinal cord	8	26.8 ± 3.3	8	140.2 ± 11.1

Since the cat is an animal of choice in investigating the functions of the CNS, the study of DNA and RNA distribution in different regions of cat’s brain has been undertaken in the belief that such data might be useful for further study of the functional alterations, possibly associated with changes of these nerve cell constituents, under various experimental conditions. Adult cats have been used in the experiments. Immediately after decapitation the brain has been removed from the skull, the pia mater has been taken off and the regions of the brain (indicated in the Table) have been separated by means of a careful dissection. Phospholipids were extracted using ethanol-ether mixture (3:1). Two extractions were done, each lasting 20 min. The residues of the brain tissue samples were washed in ethanol and then dried for 48 h *in vacuum*. All these procedures have been carried out in the cold. The extractions of DNA and RNA were performed according to the method of SCHNEIDER², and concentrations determined by CERIOTTI’s method³ for DNA, and MEJBAUM’s method⁴ for RNA.

Results are presented in the Table. As can be seen, the amounts of the DNA are of the same order in most of the regions investigated. Somewhat higher concentrations of DNA in cerebellar cortex and hypothalamus are probably due to the differences in total number of cells present in these parts of CNS. The white matter of the brain contained approximately the same amount of

¹ S.-O. BRATTGARD and H. HYDEN, *Acta radiol.* [Suppl.] **94** (1952). – L. BAKAY and O. LINDBERG, *Acta physiol. scand.* **17**, 179 (1949).
² W. C. SCHNEIDER, *J. biol. Chem.* **161**, 293 (1945).
³ G. CERIOTTI, *J. biol. Chem.* **198**, 297 (1952).
⁴ W. MEJBAUM, *Hoppe-Seylers Z.* **258**, 117 (1939).

DNA as the cerebral cortex, or some subcortical gray structures.

The distribution of RNA presents an entirely different picture. The highest concentrations were found in areas of the cerebellar and cerebral cortex, somewhat lower in caudate nucleus, thalamus and hypothalamus, and the lowest in the midbrain and more distal structures. Such a distribution of RNA apparently reflects the process of phylogenetic and ontogenetic development, and the degree of morphological and functional differentiation of the CNS. Whether the distribution pattern is due to the cytoarchitectonic differences among various regions is not clear at present. The highest concentrations of RNA in the cortical regions confirmed once again a well-known fact that they are the sites of the greatest metabolic activities.

LJ. MIHAILOVIĆ, B. D. JANKOVIĆ,
M. PETKOVIĆ, and D. MANČIĆ

Institute of Pathological Physiology, Medical School; Microbiological Institute, School of Pharmacy, University of Belgrade, and Biochemical Department, Boris Kidrič Institute for Nuclear Sciences, Belgrade (Yugoslavia), September 18, 1957.

Résumé

Chez le chat normal, la concentration d'ADN est à peu près la même dans les diverses régions examinées du système nerveux central. Par contre, la concentration d'ARN varie considérablement d'une région à l'autre. Ces résultats suggèrent que la distribution de l'ARN correspond à la différenciation morphologique et fonctionnelle des parties du cerveau.

La posizione occupata dalla fosforilcolina, citidindifosfatocolina, α -glicerilfosforilcolina e da altri corpi analoghi nel ciclo metabolico di alcuni fosfatidi encefalici

Al fine di poter precisare la posizione occupata nel ciclo metabolico dei neurofosfatidi dai singoli corpi fosforati isolati mediante la tecnica descritta in altra sede¹, è stata studiata *in vivo* la velocità di «turnover» di ciascuno di essi.

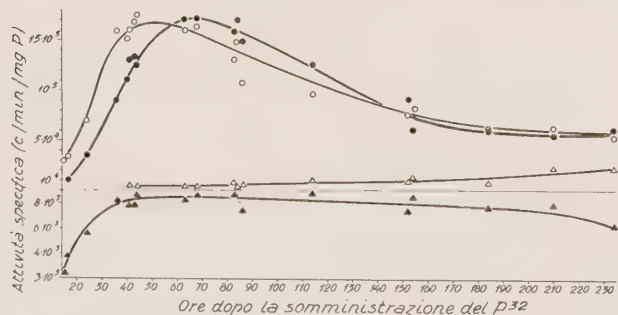


Fig. 1. Velocità di «turnover» della fosforilcolina (○), citidindifosfatocolina (●), L- α -glicerilfosforilcolina (△) e fosfatidilcolina (▲) nel sistema nervoso centrale di ratti di pochi giorni di vita. Ciascun segno indica il valore medio dell'attività specifica ottenuto da 4 esperimenti.

¹ N. MIANI, *Exper.* 14, 34 (1958).

Ratti del ceppo Wistar ricevettero per via intraperitoneale al 3° giorno di vita una singola dose di $\text{NaH}_2^{32}\text{PO}_4$ (3 $\mu\text{g/g}$ di animale) e furono sacrificati per decapitazione nell'ordine indicato nelle Figure 1-3. Ciascun segno riportato nelle figure indica il valore medio dell'attività specifica ottenuto da 4 esperimenti.

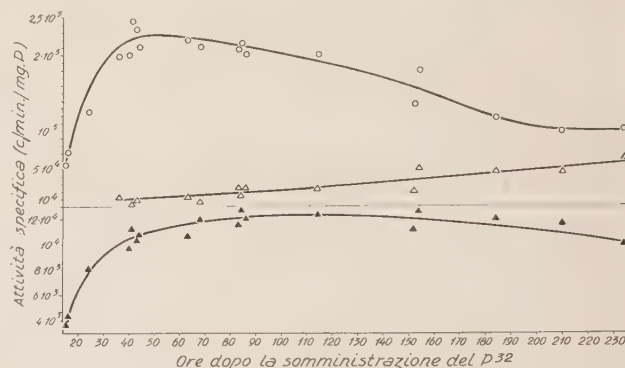


Fig. 2. Velocità di «turnover» della fosforiletanolamina (○), L- α -glicerilfosforiletanolamina (△) e fosfatidiletanolamina (▲) nel sistema nervoso centrale di ratti di pochi giorni di vita. Ciascun segno indica il valore medio dell'attività specifica ottenuto da 4 esperimenti.

Dall'esame delle curve si possono trarre le seguenti conclusioni:

1° La fosforilcolina ha la più alta velocità di «turnover» rispetto agli altri corpi fosforati contenenti colina.

2° Sulla base di presupposti teorici², la citidindifosfatocolina rappresenta l'immediato prodotto di trasformazione della fosforilcolina.

3° A sua volta la curva di attività specifica della citidindifosfatocolina e quella della fosfatidilcolina si dimostrano in rapporto quantitativo tale da lasciare ampio credito alla supposizione che il primo composto generi il secondo.

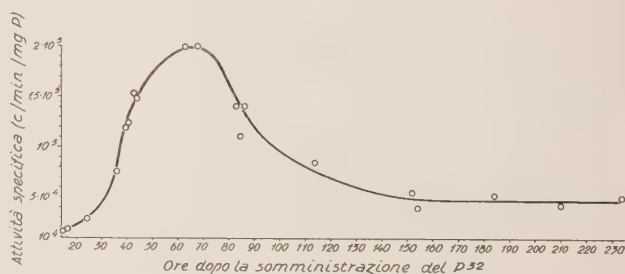


Fig. 3. Velocità di «turnover» della fosforilserina nel sistema nervoso centrale di ratti di pochi giorni di vita. Ciascun segno indica il valore medio dell'attività specifica ottenuto da 4 esperimenti.

4° L'andamento della curva di attività specifica relativa alla L- α -glicerilfosforilcolina suggerisce che essa non rappresenti il prodotto di trasformazione della fosforilcolina o della citidindifosfatocolina; può invece rappresentare il prodotto di degradazione della fosfatidilcolina o almeno di quella quota di essa metabolicamente più attiva.

5° Le curve di attività specifica della fosforilcolina, fosforiletanolamina e fosforilserina hanno un decorso fondamentalmente simile.

² D. B. ZILVERSMIT, C. ENTENMAN e M. C. FISHLER, *J. gen. Physiol.* 26, 325 (1943). - J. M. REINER, *Arch. Biochem. Biophys.* 46, 53, 80 (1953).

6° Le curve di attività specifica della L- α -glicerilfosforilcolina e L- α -glicerilfosforiletanolamina hanno pure esse un decorso simile.

7° La sintesi dei fosfatidi contenenti colina (lecitina e probabilmente i fosfosfingosidi), o etanolamina (fosfatidiletanolamine e acetalfosfatidiletanolamina) oppure serina (fosfatidilserina) si attua attraverso tappe omologhe, secondo lo schema: fosforil-base azotata $\rightarrow$ citidindifosfato-base azotata $\rightarrow$ fosfatidil-base azotata.

8° La degradazione della fosfatidilcolina e della fosfatidiletanolamina (probabilmente anche della acetalfosfatidiletanolamina) dà origine alla L- α -glicerilfosforil-base azotata corrispondente.

N. MIANI e G. BUCCIANTE

Istituto di Anatomia, Università di Padova, il 16 settembre 1957.

Summary

The rate of turnover of individual P-compounds, related to the metabolic cycle of some phosphatides, was followed *in vivo* in the brain of rats a few days old after ^{32}P administration. The phosphorylcholine is the precursor of the cytidinediphosphatecholine; this in turn may be the precursor of lecithin. The L- α -glycerylphosphorylcholine represents the degradation product of lecithin. The specific activity curves of the phosphoryl-ethanolamine and phosphorylserine are similar to that of the phosphorylcholine; likewise the L- α -glycerylphosphorylethanolamine is similar to the activity curve of the L- α -glycerylphosphorylcholine.

On the Incorporation of S^{35} -Methionine in Artificially Activated Sea Urchin Eggs

In a previous paper the results were described of experiments in which the distribution of S^{35} labelled DL-methionine among cell fractions of unfertilized and developing sea urchin eggs was studied (NAKANO and MONROY¹). In that investigation advantage was taken of a newly developed technique which allows efficient incorporation of radioisotopes in the unfertilized sea urchin eggs (NAKANO and MONROY²). It was shown that in the unfertilized egg the isotope is mainly stored in the fraction soluble in 10% trichloroacetic acid (non-protein fraction, called 'TCA-soluble fraction') while only a minor percentage is taken up by other cell components. This situation does not substantially alter upon storage of labelled unfertilized eggs up to 6 h. Upon fertilization and initiation of development, a depletion of radioactivity of this fraction takes place; this process is very rapid during the first few hours of development and then slows down progressively. At the same time a rapid uptake of isotope is observed in the mitochondria fraction; this follows an exponential course during the first 6 h of development and afterwards proceeds almost linearly.

It appeared then interesting to make a similar investigation on eggs artificially activated, in which no morphogenesis takes place.

Also, in the present investigation, S^{35} -DL-methionine has been used which was incorporated in the unfertilized eggs by the injection of 10–20 μc into the body cavity of adult females of *Paracentrotus lividus*, as previously described (NAKANO and MONROY²).

Activation was obtained by a 45 s treatment of the egg suspension with butyric acid (7 ml of 0.1 N butyric acid in 100 ml of egg suspension). The acid was then quickly neutralized with 0.7 ml of 1 N NaOH. Membrane formation soon took place. The eggs were then washed three times with sea water and finally divided into three batches which were allowed to develop under gentle shaking at room temperature (about 21°C). A sample of non-activated eggs was also collected.

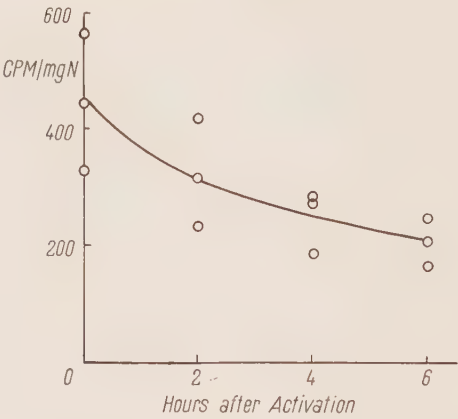


Fig. 1.—Decrease of the activity of the TCA-soluble fraction during the first 6 h after artificial activation of eggs of *Paracentrotus lividus*. The 0 time is the activity in the unfertilized eggs.

The butyric acid activation results, as it is known, in membrane formation and in a succession of monaster cycles. After a couple of hours, the eggs begin to form irregular furrows which give rise to blastomere-like formations. In the course of several hours the eggs undergo cytolysis.

In the present experiments the eggs were collected 2, 4 and 6 h after activation. The washed eggs were homogenized and the homogenate was fractionated as previously described (NAKANO and MONROY¹). Three such experiments were performed and the results are illustrated in the accompanying diagrams.

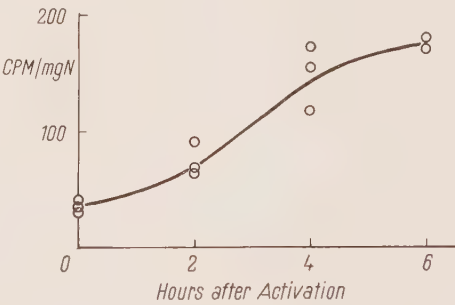


Fig. 2.—Uptake of the isotope by the mitochondria fraction of artificially activated eggs of *Paracentrotus lividus*.

The activity of the TCA-soluble fraction undergoes a decline which is rapid during the first 2 h and then proceeds more slowly (Fig. 1). The reverse is true for the mitochondria fraction in which the uptake of the isotope follows an exponential course (Fig. 2).

These results can thus be well superimposed on those obtained in the case of normally fertilized and developing eggs. This may suggest that these processes, taking place during the first few hours, which in the fertilized eggs correspond to the cleavage period, depend on activation itself rather than on morphogenesis.

¹ E. NAKANO and A. MONROY, *Exper. Cell Res.* (in press).
² E. NAKANO and A. MONROY, *Exper.* 13, 416 (1957).

This investigation was supported by Grants from Consiglio Nazionale delle Ricerche and the Rockefeller Foundation.

E. NAKANO*, G. GIUDICE, and
A. MONROY

Laboratory of Comparative Anatomy, The University
of Palermo (Italy), September 19, 1957.

Riassunto

Metionina-S³⁵ è stata incorporata in uova vergini di *Paracentrotus lividus*. In queste l'isotopo si trova per la più gran parte nella frazione solubile in acido tricloroacetico al 10%. Attivando le uova partenogeneticamente con acido butirrico si osserva una progressiva perdita di attività di questa frazione ed una rapida incorporazione nei mitocondri. L'andamento del fenomeno è del tutto identico a quello già descritto (NAKANO e MONROY) nelle uova normalmente fecondate.

* From the Biological Institute, Nagoya University, Japan. Aided by a Grant from the Rockefeller Foundation.

Efficacité de la cystéamine, administrée par ionophorèse, contre une dose focalisée de rayons X

La cystéamine et la cystamine, administrées immédiatement avant une irradiation protège la souris contre une dose de rayons X létale pour tous les témoins. Ces corps se classent parmi les meilleurs protecteurs connus jusqu'à présent¹.

Dans le cadre de nos travaux sur la radioprotection, nous avons recherché si ces substances avaient une action locale sur une irradiation locale.

Matériel et méthode. Afin d'éliminer toute action éventuelle d'un excipient, nous avons administré la cystéamine *in loco* par ionophorèse.

Le train postérieur du rat est soigneusement rasé avant l'expérience. Une solution de cystéamine à 1% imbibe une compresse entourant la cuisse droite, tandis que du liquide physiologique imbibe la compresse enserrant la cuisse gauche. Ces deux tampons sont reliés aux électrodes d'un appareil d'ionisation fonctionnant sous 3,5 mA, pendant 20 min. La cystéamine est à l'anode.

L'irradiation est effectuée immédiatement après l'ionisation, avec les constantes physiques suivantes: 50 KV, 2 mA, D.F. 4 cm, Filtre 0, débit: 2000 r/min.

La repousse des poils sert de test.

Nos expériences ont porté sur 10 rats.

Résultat.

Avec notre technique, la dose minimum épilante, sans lésions d'épidermite grave est comprise entre 800 et 1200 r. Tous nos animaux ont reçu 1000 r sur chaque cuisse.

Trois semaines après l'irradiation, la peau a terminé sa réaction épithéliale, et les zones irradiées présentent un aspect rasé légèrement luisant et sans poils, tandis qu'autour du champ réapparaît un duvet léger. Il faut

environ un mois pour que les poils repoussent en nappe régulière sur les zones rasées et non irradiées. A ce moment la zone protégée par la cystéamine est couverte d'un duvet à peine moins abondant que la peau avoisinante. La zone témoin irradiée sans protecteur reste glabre.



Ce phénomène s'accroît deux mois après l'irradiation. La zone protégée ne se distingue plus de la peau normale, tandis que sur la patte témoin persiste une zone exempte de poils (Figure).

Discussion. Il n'existe guère, dans la littérature de recherches consacrées à l'action radioprotectrice locale après application locale.

FORSSBERG² a montré l'effet protecteur de la cystéine injectée par voie intradermique contre l'épilation de la peau du cobaye; HOFFMANN³ avec la même substance, a pris comme test l'érythème; DARCIS *et al.*⁴ ont étudié l'action de la cystéamine sur la muqueuse vaginale du rat irradié par voie endocavitaire à l'aide d'un tube de Radium. L'examen des frottis leur ont permis de mettre en évidence un effet protecteur sur les cellules de cet épithélium. On pouvait se demander si la cystéamine devait être résorbée dans le torrent circulatoire pour être efficace. Nos expériences prenant comme test une zone témoin et une zone protégée chez le même animal, semblent prouver qu'il s'agit surtout d'un phénomène de radioprotection locale.

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de l'Université de Liège (Belgique), le 13 septembre 1957.

Summary

(1) Cysteamine given locally by ionisation to the skin of the rat, immediately before irradiation, protects the skin against the depilation induced by an X-ray dose of 1000 r.

(2) In our experiments, a control area is chosen on the same animal. It seems that the action of the protective substance is a local phenomenon.

² A. FORSSBERG, Acta radiol. 33, 296 (1950).

³ D. H. HOFFMANN, Strahlentherapie 96, 396 (1955).

⁴ L. DARCIS, P. HOTTERBEEX et C. ONKELINX, Exper. 12, 286 (1956).

¹ Z. M. BACQ et A. HERVE, Bull. Acad. Méd. Belg. 17, 13 (1950). Z. M. BACQ, Bull. Acad. Méd. Belg. 18, 425 (1953). - Z. M. BACQ et A. HERVE, C. R. Soc. Biol. 149, 1509 (1955). - A. HERVE et Z. M. BACQ, J. Radiol. Electrol. 33, 551 (1952).

Nachweis einer physiologischen Spontanaktivität in Einzelfasern des N. opticus der Katze

Die mit Makroelektroden von verschiedenen Stellen des visuellen Systems ableitbaren Potentialschwankungen repräsentieren die Summe von raschen Aktivitätsänderungen, wie sie in zahlreichen Einheiten bei plötzlicher Änderung der retinalen Beleuchtung eintreten (on- bzw. off-Effekte). Eine Spontanaktivität im Sinne einer dauernden Impulsentladung bei vollkommener Dunkelheit kann dagegen nur mit Mikroelektroden registriert werden. Derartige Spontanentladungen wurden sowohl bei retinalen Ganglienzellen (GRANIT¹, KUFFLER²) als auch bei Ganglienzellen des visuellen Cortex (JUNG³) nachgewiesen und waren im Zusammenhang mit Problemen des retinalen «Eigenlichts» Gegenstand eingehender theoretischer Betrachtungen (BARLOW⁴). Die Registrierung der Spontanaktivität retinaler bzw. kortikaler Ganglienzellen setzt voraus, dass die Mikroelektrode in das betreffende Gewebe eingestochen und möglichst nahe an die zu untersuchende Zelle gebracht wird. Im Hinblick auf den dadurch bedingten Insult ist die Möglichkeit nicht auszuschliessen, dass lokale Effekte an der registrierten Aktivität beteiligt sind. Bei den in vorliegenden Untersuchungen registrierten Spontanentladungen von Einzelfasern des N. opticus konnte dieser Einwand widerlegt und damit die physiologische Natur der visuellen Spontanaktivität bewiesen werden.

Die Untersuchungen wurden am «hängenden Gehirn» nach SCHUBERT⁵ durchgeführt, da bei dieser Präparation dank Erhaltung einer normalen Blutversorgung des Sehnerven stabile Versuchsbedingungen gewährleistet sind. Entsprechend den Erfahrungen von THOMSON⁶ wurden zur Ableitung von Spike-Potentialen Metallmikroelektroden von 3–7 μ Durchmesser verwendet; elektrolytisch zugespitzter Silberdraht (BURTT und CATTON⁷) wurde unter mikroskopischer Kontrolle in Mikropipetten eingeschmolzen, die nach dem Verfahren von WEALE⁸ hergestellt wurden. Die Mikroelektrode wurde mittels Zeißschem Mikromanipulator in den N. opticus knapp vor dem Chiasma eingestochen, Bezugs- und Erdelektrode befanden sich am Rand der Operationswunde bzw. am Scheitel. Verstärkung und Registrierung erfolgte mit Grass Pre-amplifier P-4 (mit vorgeschalteter Kathodenfolgerstufe), Kathodenstrahl-oszillograph Philips GM 3156 (Zeitkonstante auf 1 ms herabgesetzt) und Photokymographion. Ein einfaches Gerät ermöglichte die Erzeugung von Lichtreizen variabler Intensität und Dauer (Reizfeld 30 cm vor dem Auge, 3 cm $\varnothing$; Leuchtdichte maximal 80 000 asb, abstufbar durch Neutralfilter; Reizzeitbegrenzung mittels Compurverschluss). Der intraokulare Druck konnte durch eine in die Vorderkammer eingeführte Lebersche Kanüle mit angeschlossener Druckeinrichtung (Mariottesche Flasche mit Heparin-Ringer-Lösung, Hg-Manometer) kontrolliert bzw. variiert werden.

Mit der angewandten Methode gelang es, Impulse vom intrakraniellen Abschnitt des N. opticus abzuleiten, wo-

bei die Retina vollkommen intakt blieb. Hinsichtlich Form und Polarität glichen die abgeleiteten Impulse weitgehend den von THOMSON⁶ im Sehnerven des Kaninchens nachgewiesenen Spikes. Durch stufenweises

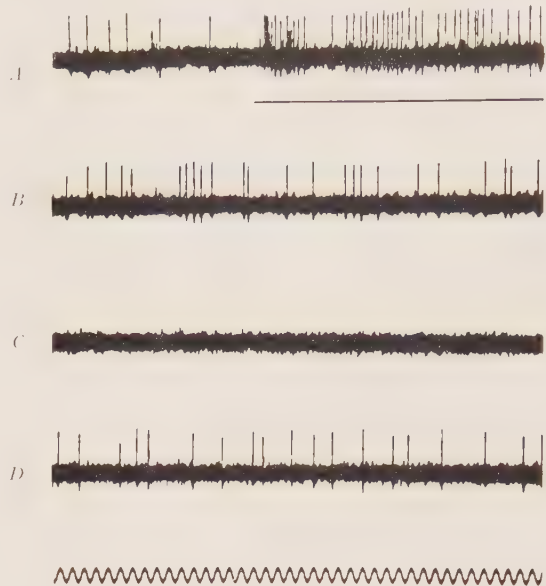


Abb. 1. Aktionspotentiale einer Fasereinheit im intrakraniellen Abschnitt des N. opticus der Katze. On-Effekt bei Belichtung der Retina (A). Die nach einstündiger Dunkeladaptation registrierte Spontanaktivität (B) wird durch eine 60 s dauernde retinale Ischämie ausgelöscht (C) und ist 5 min nach Ende der intraretinalen Blockade wieder im ursprünglichen Ausmass nachweisbar (D). Zeitmarkierung: 50 Hz.

Verschieben der Mikroelektrode konnten die Entladungen einzelner Einheiten isoliert und bei günstigen Versuchsbedingungen stundenlang beobachtet werden. Die Reaktion der verschiedenen Fasereinheiten auf Lichtreize zeigte die für retinale Neurone charakteristischen Typenunterschiede (on- bzw. off-Entladungen).

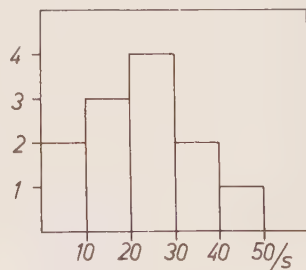


Abb. 2. Häufigkeitsverteilung der Spontanfrequenzen von 12 Fasereinheiten des N. opticus nach einstündiger Dunkeladaptation.

Gemeinsames Merkmal aller untersuchter Faser-einheiten war eine ausgeprägte Spontanaktivität, die sich in dauernden Impulsentladungen bei kompletter Dunkelheit manifestierte. Dass diese Aktivität nicht durch die Anwesenheit der Mikroelektrode im Nerv bedingt war, zeigte ihr rasches Verschwinden bei Erzeugung einer retinalen Ischämie durch plötzliche Steigerung des intraokularen Druckes auf 200 mm Hg. Diese Unterdrückung der Spontanaktivität war bei kurzer Dauer der retinalen Ischämie vollkommen reversibel und beliebig oft wiederholbar (Abb. 1). Über Einzelheiten der Erscheinung (Lähmungs- und Erholungs-

¹ R. GRANIT, *Sensory mechanisms of the retina* (Oxford University Press, London 1947).
² S. W. KUFFLER, *J. Neurophysiol.* 16, 37 (1953).
³ R. JUNG, *EEG Clin. Neurophysiol. Suppl.* 4, 57 (1953).
⁴ H. B. BARLOW, *J. Physiol.* 136, 469 (1957).
⁵ G. SCHUBERT, *v. Graefes Arch. Ophthalm.* 154, 167 (1953).
⁶ L. C. THOMSON, *J. Physiol.* 119, 191 (1953).
⁷ E. T. BURTT und W. T. CATTON, *J. Physiol.* 133, 68 (1956).
⁸ R. A. WEALE, *J. Physiol.* 112, 4 P (1951).

zeiten) wird gesondert berichtet werden. Für die vorliegende Fragestellung ist lediglich die Tatsache entscheidend, dass die nahe dem Chiasma abgeleitete Spontanaktivität des N. opticus durch ischämische Blockade der intraretinalen Strecke des Neurons bzw. seiner präsynaptischen Verbindungen ausgelöscht wird und daher nachweislich nicht an der Ableitstelle entsteht.

Die Frequenz der Spontanentladungen zeigte eine bemerkenswerte Varianz. Bei 12 einwandfrei isolierten Einheiten wurden nach jeweils einstündiger Dunkeladaptation die in Abbildung 2 dargestellten Impulsfrequenzen gemessen (Mittelwert $\pm$ Standardabweichung 22 ± 11 Impulse/s). Sie stimmen größenordnungsmässig mit den bei retinalen und kortikalen Ganglienzellen beobachteten Spontanfrequenzen gut überein (retinale Spontanfrequenz etwa 20–30/s nach KUFFLER², Variationsbreite der Spontanfrequenz lichtaktivierter kortikaler Einheiten 2–40/s nach JUNG und BAUMGARTNER⁹).

Der eindeutige Nachweis einer physiologischen Natur der Spontanaktivität im N. opticus erscheint insofern von Interesse, als dieses Phänomen grundsätzlich bei jeder Sehtheorie berücksichtigt werden muss.

H. BORNSCHEIN

Physiologisches Institut der Universität Wien, den 28. September 1957.

Summary

Spike potentials were recorded with metal micro-electrodes from single fibers in the intracranial part of the cat's optic nerve with the retina left completely intact. All units studied as yet showed a marked spontaneous activity irrespective of differences in their response to light stimuli. The spontaneous activity in the intracranial part of the optic nerve could be suppressed reversibly by increasing the intraocular pressure up to 200 mm Hg. Thus spontaneous activity has been verified as a normal feature of the retina. A spontaneous firing rate of 22 ± 11 /s after 1 h dark adaptation was found in altogether 12 well-isolated fiber units.

⁹ R. JUNG und G. BAUMGARTNER, Pflügers Arch. ges. Physiol. 261, 434 (1955).

Studies of the Differences of Osmotic Pressure between the Aqueous Humor and the Serum in some Species of Animals

The first attempts to measure differences in osmotic pressure between the plasma and the aqueous humor were made in 1927 by DUKE ELDER¹; he determined the modifications of electrical conductivity of the two fluids separated by a collodion membrane, and stated at the time that they were in osmotic equilibrium. However, subsequent investigations, by GILMANN and YUDKIN² and by BENHAM *et al.*³, led to the definite conclusion that aqueous humor is hypertonic to serum, with a difference of 5 mM NaCl.

¹ W. S. DUKE ELDER, J. Physiol. 62, 315 (1927).

² A. GILMANN and A. M. YUDKIN, Amer. J. Physiol. 104, 235 (1933).

³ G. H. BENHAM, H. DAVSON, and W. S. DUKE ELDER, J. Physiol. 89, 61 (1937).

The findings have since been confirmed by ROEPKE and HETHERINGTON⁴, BARANY⁵, KINSEY⁶, and SCHAEFFER⁷, and at the present time it is generally accepted that stabilization of this osmotic gradient is of considerable importance in the mechanism of intraocular pressure.

Although now several authors have abundantly proved that the osmotic pressure of the aqueous humor is higher than that of the plasma in man, rabbits, dogs, and cats by some 2–4%, and equivalent to a hydrostatic pressure of some 90–180 mmHg, and normal intraocular pressure is only 20–25 mmHg, the hydrostatic potential of this difference in osmotic pressure does not manifest itself, and as things stand now, no satisfactory explanation of this can be given. HARRIS⁸ is very probably right in his assumption that the flow of aqueous humor in the eye is so rapid that osmotic and hydrostatic balancing does not take place.

DUKE ELDER¹, ROEPKE⁴, BARANY⁵, and KINSEY⁶ carried out their observations using the thermo-electric method of BALDES, which measures the depression of the water vapour in the fluids under study; a later modification by KINSEY⁶ of this method, eliminating some disadvantages of the thermo-couples, was used to study modifications of the osmotic pressure under some experimental conditions. ROEPKE and HETHERINGTON⁴ did not find any differences of the molar concentration of the aqueous humor in subjects with glaucoma simplex; BARANY⁵ found no differences of osmotic pressure in the aqueous humor of rabbits after unilateral ligation of the carotid, although he saw a decrease of ascorbic acid and an increase of lactic acid and CO₂. The same author observed a slight decrease of osmotic pressure after instillation of eserine and atropine.

More recently, SCHAEFFER⁷ made some studies of the osmotic pressure of the lacrimal fluid, in which he applied a very simple method for which no complicated apparatus is needed and which makes it possible to carry out determinations even in quantities of fluid of less than 0.1 ml with a precision of $\pm 0.01 M$. In an attempt to contribute to the solution of this interesting problem of the possible modifications of the osmotic pressure under normal and pathological conditions, I have started preliminary studies of the aqueous humor and serum of various species of animals, and of the aqueous humor and serum of man under normal conditions.

Experimental Part. — The method used, a modification by NIEDERL and LEVY⁹ of the well-known method of BARGER, is based on the fact that in a closed system, in which two fluids contained in capillary tubes are compared, a modification of the proportion of the volumes is due to a difference in the distribution of the water vapour in the two solutions. The tendency of the system is towards osmotic equilibrium, and the solution with the highest molarity will increase in volume and that with the lowest molarity will decrease in volume until an equilibrium is attained.

When this method is used, two capillary tubes are filled with the solutions in question, closed at one end and placed in an outer tube in such a way that the two menisci are at the same level; the pressure in the outer tube is decreased by some 15 mmHg, after which the

⁴ R. R. ROEPKE and W. A. HETHERINGTON, Amer. J. Physiol. 130, 340 (1940).

⁵ E. H. BARANY, Acta physiol. scand. 13, 81 (1947).

⁶ V. E. KINSEY, J. gen. Physiol. 34, 389 (1951).

⁷ A. I. SCHAEFFER, Arch. Ophthalm., N. Y. 43, 1026 (1950).

⁸ I. E. HARRIS, Eye Digest, May 1952.

⁹ I. B. NIEDERL and A. M. LEVY, Science 92, 2384 (1940).

Animals	Number of samples	Aqueous humor	Serum	Difference
Horse	4	161.4	158.2	+ 3.2
Cow	4	162.0	158.6	+ 3.4
Sow	3	160.6	159.5	– 1.4
Dog	8	159.6	157.4	+ 2.2
Rabbit	12	157.2	156.8	+ 1.4
Guinea-pig . . .	12	156.3	156.8	– 0.5
Man	2	160.4	158.3	+ 2.1

tube is sealed. In the original method, the system was then left to stand at room temperature for 4 days, after which the difference in level between the two menisci was read. Since, however, the prolonged interval made it possible for the fluids to undergo chemical changes which might influence the results, the original method has been modified by NIEDERL and LEVY in such a way that the time required for equilibrium is decreased, at the same time decreasing the space to be evacuated in which evaporation and osmotic equilibrium occur.

It may be presumed that the value of exchange in the time *t* of the quantity of fluid evaporated, *m*, equals a factor *α* minus a quantity which is inversely proportional to the volume *V*. Expressed as a differential equation, this reads:

$$\frac{dm}{dt} = \alpha - \frac{\gamma}{V} m.$$

(1)

The integral solution is:

$$m = \frac{aV}{\gamma} (1 - e^{-\frac{\gamma}{V}t}).$$

(2)

Consequently, when all other conditions remain the same, decrease of the volume decreases the time needed for an equilibrium to be attained.

In my investigation, 15 mmHg was evacuated, after which the system was left to stand at room temperature for 12 h; and the difference in level between the two menisci was then read. If the two menisci were at the same level, the fluids had the same osmotic pressure; if not, they were hetero-osmotic.

Using slight magnification, one is able to demonstrate differences of 0.01 *M* (about 0.06 g NaCl/100 ml).

Previous to the tests of the aqueous humor, a series of tests had been made in which standard solutions of NaCl of 0.83% and of 0.98% were compared; the values for the aqueous humor were expressed in terms of isotonicity with solutions of NaCl in *mM*/kg H₂O.

This method, which is very simple and can be repeated at will, makes it possible to carry out determinations in quantities of aqueous humor of less than 0.1 ml.

In my present preliminary investigations, I have determined the osmotic pressure of the aqueous humor in some species of animals in relation to the osmotic pressure of the serum. The two fluids were taken from the same animal, at practically the same moment. In the Table, the results obtained in the various species examined are given.

Scrutiny of this table reveals that the hypertony of the aqueous humor over the serum is a constant finding in all the species examined, with the exception of guinea-pigs.

The fact that in guinea-pigs the aqueous humor is hypotonic with regard to the plasma is not surprising if

one remembers the recent observations by DAVSON¹⁰, who has demonstrated a difference of the ratios of concentration of bicarbonates in aqueous humor and plasma of various species of animals, and a variability of the ratio of concentration of the chlorides. The guinea-pig, in particular, shows a deficiency of anions (chlorides and bicarbonates) and it is this deficiency which explains the hypotony of the aqueous humor which makes it possible to maintain a rate of flow of the endocular fluid compatible with a relatively normal intra-ocular pressure.

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July 29, 1957.*

Riassunto

L'autore ha eseguito determinazioni della pressione osmotica dell'U. Acqueo e siero in alcune specie animali col metodo di NIEDERL e LEVY. In tutte le specie animali ha riscontrato sempre un'ipertonía dell'Acqueo rispetto al siero di circa 2 *m*/*M*.

Soltanto nella cavia l'acqueo è leggermente ipotonico in rapporto al siero. L'autore spiega questa eccezione con la deficienza di anioni (cloruri e bicarbonati riscontrata nell'acqueo di cavia).

¹⁰ H. DAVSON, *Physiology of the ocular and cerebrospinal fluids* (Churchill 1956).

A Comparison of the Effect of Potassium Ions
on the Metabolism of Retina and Brain Cortex
Slices *in vitro*

It is generally accepted that potassium ions play an important role in the functional metabolism of nervous tissue¹. Further information on the metabolic response of nervous tissue to increased K⁺ concentration *in vitro* therefore appears to be desirable.

Comparing the available evidence on the metabolism of brain cortex slices and retina *in vitro*, it will be noted that an increased concentration of K⁺ in glucose-containing saline brings about an increased oxygen consumption and aerobic glycolysis of incubated brain cortex slices², while no such response has been observed in retina³. The results of TERNER *et al.*⁴ indicate that the same mechanism applies to the movement of K⁺ in both retina and brain cortex slices. It is also known that on incubation retina releases considerable amounts of ammonia⁵, in a similar way as do brain cortex slices. Experiments were published supporting the view that ammonia formation is closely connected with the functional metabolism of retina as well as of other nervous

¹ A. L. HODGKIN, *Biol. Rev.* 26, 339 (1951). – R. D. KEYNES and P. R. LEWIS, in *Neurochemistry* (Ed. by K. A. C. ELLIOTT, J. H. PAGE, J. H. QUASTEL; Ch. S. Thomas, Springfield, Illinois 1955).
² C. A. ASHFORD and K. C. DIXON, *Biochem. J.* 29, 157 (1935). – A. CANZANELLI, G. ROGERS, and D. RAPPORT, *Amer. J. Physiol.* 135, 309 (1942). – M. N. LIPSETT and F. CRESCITELLI, *Arch. Biochem.* 28, 329 (1950). – H. McILWAIN, *Biochem. J.* 52, 289 (1952). – M. B. R. GORE and H. McILWAIN, *J. Physiol.* 117, 471 (1952). – Y. TSUKADA and G. TAKAGAKI, *Nature* 175, 725 (1955).
³ F. DICKENS and G. D. GREVILLE, *Biochem. J.* 29, 1468 (1935).
⁴ C. TERNER, L. V. EGGLESTON, and H. A. KREBS, *Biochem. J.* 47, 139 (1950).
⁵ O. WARBURG, K. POSENER, and E. NEGELEIN, *Biochem. Z.* 152, 309 (1924).

Oxygen consumption, ammonia formation and aerobic lactic acid formation in pig retina and guinea-pig brain cortex slices in salines of different K^+ concentrations with and without glucose. Each vessel contained 4 ml saline and tissue. The average wet weight of tissue was 129.3 ± 15.4^a mg retina or 129.1 ± 10.2 mg^a brain cortex slices. Each value in the Table is the average of 6 experiments (unless otherwise stated). O_2 as gaseous phase, 37° C. The results are expressed in $\mu M/g$ wet weight of tissue.

	a					b					c ^b					d ^d				
	O_2 used (1 h)					O_2 used (4 h)					NH_3 formed (4 h)					Lactic acid formed (1 h)				
No. of medium	1	2	3	4		1	2	3	4		1	2	3	4	5	1	2	3	4	5
$K^+ mM/l$	6.25	131.5	6.25	131.5		6.25	131.5	6.25	131.5		6.25	131.5	6.25	131.5	TCA	6.25	131.5	6.25	131.5	TCA
Glucose mM/l	0	0	10	10		0	0	10	10		0	0	10	10	added at 0 time t_0	0	10	10	10	added at 0 time t_0
Pig retina	53.3 $\pm 5.5^a$	30.1 ± 3.4	45.4 ± 4.7	35.5 ± 2.5		130.6 ± 16.9	84.0 ± 10.1	151.5 ± 12.9	114.5 ± 9.3		14.6 ^c ± 2.1	17.5 ± 3.3	17.5 ± 3.3	17.9 ± 2.8	6.2 ± 1.3	113.1 ± 2.8	89.4 ± 9.0	113.1 ± 2.8	89.4 ± 9.0	7.0 ± 1.3
Guinea-pig brain cortex slices	64.3 ± 1.7	46.0 ± 2.8	61.0 ± 4.0	78.9 ± 5.1		141.1 ± 4.6	75.8 ± 6.2	224.8 ± 9.3	252.3 ± 20.1		29.8 ± 1.2	23.4 ± 2.3	23.4 ± 2.3	26.8 ± 2.1	8.6 ± 0.9	62.6 ± 10.1	111.7 ± 15.3	62.6 ± 10.1	111.7 ± 15.3	10.4 ± 3.9

a $\sum J^2/n(n-1)$.

b A considerable part of ammonia formed during incubation of both tissues is bound by endogenous glutamic acid as glutamine, if glucose is used as substrate⁹. Ammonia was therefore estimated in the TCA extract of the tissue after hydrolysing the extract for 75 min at 70°C. The values thus present the sum of free and bound TCA-soluble ammonia. This procedure makes a direct comparison of ammonia formation in glucose-free and glucose-containing salines possible.

c Average of 11 experiments.

d In experiments recorded in section d 100-120 mg wet weight of tissue were used in each vessel. Each value is an average of 5 experiments.

tissue⁶. We have recently observed⁷ that ammonia formation in guinea-pig brain cortex slices, incubated in glucose-free saline, was inhibited by increased concentrations of K^+ . The question arose whether K^+ also inhibits ammonia formation in retina.

Experiments were performed on guinea-pig brain cortex slices and pig retina. The preparation of brain cortex slices was described earlier⁷. Pig retinas were prepared as described by TERNER *et al.*⁴, with small modifications. The slices, or retinas, were then incubated in Warburg flasks. Each experiment was performed simultaneously in five media: Medium 1: Phosphate saline with a trace of bicarbonate, no substrate added⁷; Medium 2: K^+ concentration increased by completely replacing NaCl with KCl; Medium 3: same as medium 1, but 10 mM/l glucose added; Medium 4: same as medium 2, but 10 mM/l glucose added. After 4 h incubation at 37° with O_2 as the gaseous phase, the vessels were detached from the manometers and the reaction stopped by addition of 1 ml 50% trichloroacetic acid (further TCA). To each experimental series, a fifth vessel was included containing 4 ml of medium 1 and tissue, but TCA was added before the tissue was introduced (Medium 5). During the incubation oxygen utilization was measured (0.2 ml 20% KOH and a folded filter-paper in the centre well of the vessels). In the TCA-extracts ammonia was estimated (for details see ⁷). In a series of experiments lactic acid formed was also determined⁸ after treatment of the TCA-extracts with copper lime.

The results of these experiments, recorded in the Table, permit the following conclusions:

(1) An increase of the K^+ concentration in the medium in absence of added substrate decreases oxygen consumption (Table a and b, columns 2) and ammonia formation (Table c, column 2) in both pig retina and guinea-pig brain cortex slices. Addition of glucose increases oxygen consumption during 4 h incubation (Table b, column 3), but decreases ammonia formation in both tissues studied (Table c, column 3).

(2) In both pig retina and guinea-pig brain cortex slices ammonia formation is inhibited by addition of glucose or by an increase of K^+ concentration in the medium. The inhibitory effects of K^+ and glucose on ammonia formation are not additive. In presence of glucose K^+ exerts no further influence on ammonia formation (Table c, column 4).

(3) An increase of K^+ concentration in the presence of glucose brings about a decrease of both oxygen consumption and aerobic lactic acid formation in pig retina; on the other hand, in guinea-pig brain cortex slices oxygen consumption and aerobic lactic acid formation are increased under these conditions (Table d).

Thus both tissues show a marked difference in their metabolic response to increased K^+ concentration as far as oxygen utilization and aerobic lactic acid formation are concerned, but behave similarly as far as ammonia formation is concerned.

⁶ H. RÖSCH und W. TEKAMP, Z. physiol. Chem. 175, 158 (1928).

⁷ R. VRBA, J. FOLBERG, and V. KANTÚREK, Nature 179, 470 (1957); J. Neurochem. 2 (in press).

⁸ S. B. BARKER and W. H. SUMMERSON, J. biol. Chem. 133, 535 (1941).

⁹ H. A. KREBS, Biochem. J. 29, 1951 (1935).

¹⁰ D. RICHTER and R. M. C. DAWSON, J. biol. Chem. 176, 1199 (1948). - M. M. HARRIS, J. clin. Invest. 22, 569 (1943).

Acknowledgements. We wish to thank Dr. A. KLEINZELLER for criticism and helpful advice. The technical assistance of Mrs. ROSA EHRMANN is also gratefully acknowledged.

R. VRBA and JAROSLAVA FOLBERGR

Institute of Industrial Hygiene and Occupational Diseases, Prague, July 30, 1957.

Zusammenfassung

Der Einfluss einer erhöhten Kalium-Ionenkonzentration auf den *in vitro*-Metabolismus von Schweine-Retina und Gehirnrindenschnitten des Meerschweinchens wird verglichen. Die höhere Kalium-Ionenkonzentration im Medium bewirkt im ersten Falle eine Verminderung, im zweiten dagegen eine Steigerung von Sauerstoffaufnahme und anaerober Milchsäurebildung. Bei beiden Geweben wird durch die vermehrte Kalium-Ionenkonzentration die Bildung von Ammoniak gehemmt.

Corticotrophin-Releasing Action of Adrenaline, Serotonin and Pitressin

There is much evidence that the secretion of ACTH is controlled by a hypothalamic neuro-humor. The nature of this corticotrophin-releasing factor (CRF) remains obscure. Several substances have been suggested as possible transmitters. Among these are adrenaline¹, serotonin², and vasopressin³ or a vasopressin-contaminant⁴.

As the CRF must be able to stimulate the pituitary gland directly, the effect of these substances was investigated in animals in which hypothalamic functions were inhibited.

Albino rats were injected with 30 µg adrenaline intraperitoneally, 25 µg serotonin picrate⁵, 0.2 I.U. Pitressin (Parke, Davis) or 0.2 ml saline intravenously per 100 g body-weight respectively. The release of ACTH was measured by the adrenal ascorbic acid depletion; the rats were sacrificed by decapitation 1½ h after the administration of the compounds, and the ascorbic acid content of both adrenals was determined according to SAYERS *et al.*⁶.

The first experiment was carried out with normal male rats weighing 150–180 g. As may be seen from the Table, the adrenal ascorbic acid content of rats treated with any of the compounds differs statistically significant from that of the saline-treated rats.

In the second experiment, with male rats weighing 150–180 g, a corticoid was used as a hypothalamic

Treatment	Adrenal ascorbic acid content		
	None	Prednisolone	Lesioned
Pitressin . .	274 (± 6.5)*†	438(±10.2)†	316(±16.6)†
Adrenaline .	294 (± 8.9)†	514(±16.2)	380(±18.0)
Serotonin . .	295 (± 10.8)*†	511(±10.6)	401(±17.4)
Saline . . .	349 (± 10.0)	525(± 7.2)	396(±15.0)

The data are given in mg ascorbic acid / 100 g adrenal tissue, with the standard error of the mean between brackets.

† statistically significant decrease as compared with saline-treated controls.

* in these cases 13 rats are used; in all other cases 14.

blocking agent. 5 mg Prednisolone⁷ per 100 g body-weight was injected subcutaneously 24 h prior to the administration of the compounds under investigation. The Table demonstrates that the effect of both adrenaline and serotonin on the adrenal ascorbic acid content is inhibited under these conditions, whereas Pitressin still induces a statistically significant ascorbic acid decrease.

The last experiment was performed in hypothalamic-lesioned rats. In this case female rats weighing 190–210 g were used. Electrolytic lesions were made by means of a modified stereotactic instrument after GREER *et al.*⁸. A detailed description of the lesion technique is given elsewhere⁹. Lesions were placed in the posterior part of the eminentia mediana, interrupting the hypothalamo-hypophysial connections.

As is demonstrated in the Table, serotonin and adrenaline again failed to show ACTH-releasing activity in this experiment. On the other hand Pitressin is able to activate the hypophysis-adrenal axis.

The results reported here do not support the view that either adrenaline or serotonin would exert a specific action in the mechanism of the ACTH secretion from the adenohypophysis. The demonstration of the pituitary activation by Pitressin in Prednisolone-treated and hypothalamic-lesioned rats is in accordance with the findings of several authors¹⁰. Whether this effect is due to the true vasopressin or to another polypeptide contaminating Pitressin is not decided by these experiments.

P. G. SMELIK and D. DE WIED

Department of Pharmacology, Rijksuniversiteit Groningen (Holland), August 19, 1957.

Zusammenfassung

Der Einfluss von Serotonin, Adrenalin und Pitressin auf den Mechanismus der ACTH-Ausschüttung durch den Hypophysenvorderlappen wurde unter verschiedenen experimentellen Bedingungen untersucht.

Bei normalen Ratten zeigten alle geprüften Pharmaka auf die Adenohypophyse eine stimulierende Wirkung, welche nach der Abnahme des suprarenalen Ascorbinsäuregehaltes beurteilt wurde.

¹ J. E. MARKEE, J. W. EVERETT, and C. H. SAWYER, *Rec. Progr. Horm. Res.* 7, 139 (1952). – C. N. H. LONG, *Ciba Coll. Endocrinol.* 4, 139 (1952).

² A. BERTELLI, G. CANTONI, and L. MARTINI, *Atti Soc. lombarda Sci. med. biol.* 9, 10 (1954). – H. MOESSATCHE and N. A. PEREIRA, *Naturwissenschaften* 43, 517 (1956).

³ A. FRAJA and L. MARTINI, *Arch. int. Pharmacodyn.* 93, 167 (1953). – I. A. MIRSKY, M. STEIN, and G. PAULISCH, *Endocrinology* 55, 28 (1954). – S. M. McCANN, *Endocrinology* 60, 665 (1957).

⁴ M. SAFFRAN, A. V. SCHALLY, and B. G. BENFEY, *Endocrinology* 57, 439 (1955). – R. GUILLEMIN, W. R. HEARN, W. R. CHEEK, and D. E. HOUSHOLDER, *Fed. Proc.* 15, 84 (1956).

⁵ Kindly supplied by Dr. H. D. MOED, Philips-Roxane Ltd., Weesp, Holland.

⁶ M. A. SAYERS, G. SAYERS, and L. A. WOODBURY, *Endocrinology* 42, 379 (1948).

⁷ Supplied by Merck, Sharp & Dohme Research Laboratories, Haarlem, Holland, through the courtesy of Mr. J. BARENS.

⁸ M. A. GREER, C. MENCKEN, and R. K. T. BOLEN, *Proc. Soc. exp. Biol. Med.* 89, 480 (1955).

⁹ P. R. BOUMAN, J. H. GAARENSTROOM, P. G. SMELIK, and D. DE WIED, *Acta physiol. pharm. Néerl.* 6, 368 (1957).

¹⁰ S. M. McCANN and J. R. BROBECK, *Proc. Soc. exp. Biol. Med.* 87, 318 (1954). – J. C. PORTER and H. W. RUMSFIELD, *Endocrinology* 58, 359 (1956). – R. GUILLEMIN and B. ROSENBERG, *Endocrinology* 57, 599 (1955).

Bei Tieren mit Prednisolon-Vorbehandlung oder mit einer Elektrokoagulation im hypophysennahen Bezirk des Hypothalamus konnte nur mit Pitressin eine ACTH-Sekretion ausgelöst werden.

Die Verfasser schliessen daraus, dass im Gegensatz zu Pitressin weder Serotonin noch Adrenalin einen sogenannten „Neuro-transmitter“ darstellen.

De l'action radio-protectrice exercée sur la muqueuse rectale par la cystéamine administrée par voie générale et par la cystamine administrée par voie intra-rectale

I. Introduction

R. M. GRAHAM¹ a montré qu'il existe chez la femme une relation étroite entre la radio-sensibilité de la muqueuse vaginale normale et celle d'un cancer cervico-utérin. Les travaux de MALONEY², LIMBURG *et al.*³, BESSERER et SMOLKA⁴ et RUMMEL⁵ vinrent jeter un doute sur l'exactitude de cette constatation. Néanmoins, les recherches ultérieures des GRAHAM (R. M. GRAHAM⁶, J. B. GRAHAM⁷, R. M. et J. B. GRAHAM⁸), ainsi que celles de CUYLER⁹, de SHIER¹⁰ et surtout de NIELSEN¹¹,

¹ R. M. GRAHAM, Surg. Gynec. Obstet. 84, 153 (1947); 84, 166 (1947).

² G. C. MALONEY, Amer. J. Obstet. Gynec. 60, 533 (1950).

³ H. LIMBURG, J. H. NAPP et V. WILBRAND, Geburtsh. u. Frauenheilk. 12, 723 (1952).

⁴ G. BESSERER et H. SMOLKA, Strahlentherapie 60, 533 (1952).

⁵ A. RUMMEL, Zbl. Gynäk. 75, 1541 (1953).

⁶ R. M. GRAHAM, Surg. Gynec. Obstet. 93, 767 (1951).

⁷ J. B. GRAHAM, Surg. Gynec. Obstet. 37, 331 (1953).

⁸ R. M. GRAHAM et J. B. GRAHAM, Cancer 8, 59 (1955).

⁹ W. K. CUYLER, *Proceedings of the Symposium on Exfoliative Cytology*, Oct. 23–24, 1951 (New York-American Cancer Society Inc. 1953), p. 130, Disc. p. 136.

¹⁰ C. B. SHIER, Amer. J. Obstet. Gynec. 67, 286 (1954).

¹¹ A. M. NIELSEN, Acta radiol. 37, 479 (1952).

de KOHN¹² et de KJELLGREN¹³ confirmèrent les premiers travaux de R. M. GRAHAM. Cet auteur a en outre constaté que la testostérone augmente la radio-sensibilité de la muqueuse vaginale des femmes porteuses d'un cancer du col.

De notre côté (DARCIS¹⁴) nous avons étudié expérimentalement l'action des différents systèmes endocriniens sur la radio-sensibilité de la muqueuse vaginale de la rate et nous avons entre autres confirmé l'action radio-sensibilisatrice de la testostérone.

Il ressort clairement de ces différents travaux qu'il est dès à présent permis d'envisager, en clinique humaine, de modifier dans un sens positif ou négatif, la radio-sensibilité des tissus destinés à subir une irradiation intensive.

Dans le présent travail, nous avons tenté de déterminer le degré de la radio-protection exercée au niveau de la muqueuse rectale, irradiée par le Radium, d'une part par la cystéamine introduite dans la cavité péritonéale, d'autre part par la cystamine administrée dans la lumière du rectum.

II. Techniques et méthodes

Nous avons utilisé des rats blancs femelles de l'élevage sélectionné du C.A.C. de Liège. Le rectum des animaux (maintenus sous anesthésie générale pendant toute la durée de l'expérience) est soumis à l'action d'une tube de 50 mg de Radium, filtré par 1 mm de Platine, et laissé en place pendant 4 h.

La cystéamine a été injectée dans la cavité péritonéale, immédiatement avant le début de l'irradiation et à la

¹² G. KOHN, Acta radiol. 41, 446 (1954).

¹³ O. KJELLGREN, *Second Annual Meeting of the Inter-Society Cytology Council* (Boston 1954), p. 77.

¹⁴ L. DARCIS, P. HOTTERBEECH et C. ONKELINX, Exper. 12, 286 (1956). – L. DARCIS, C. ONKELINX et P. HOTTERBEECH, Ann. Endocr., Paris 17, 302 (1956). – L. DARCIS, Ann. Endocr., Paris 17, 462 (1956); *Influence des glandes endocrines sur la sensibilité de la muqueuse vaginale de la rate aux radiations* (Ed. Derouaux, Liège 1956); Ann. Endocr., Paris (sous presse). – L. DARCIS et P. HOTTERBEECH (en préparation).



Fig. 1: Frottis rectal normal

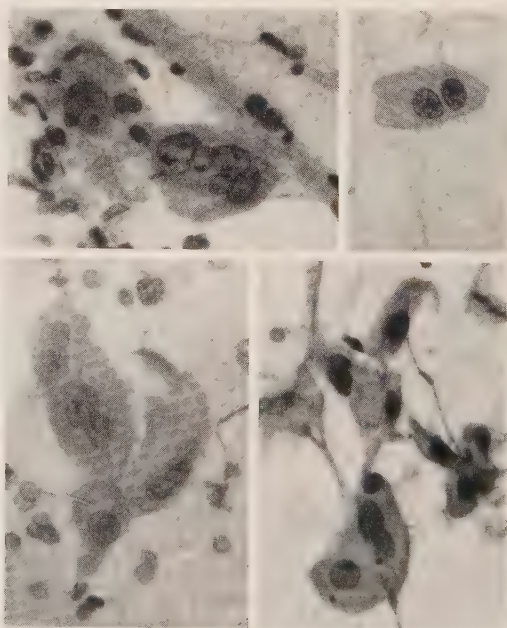


Fig. 2–5: Frottis rectal après irradiation locale

dose de 15 mg. La cystamine a été introduite dans le rectum au moyen d'un petit «Baxter» relié à un fin tube de polytène; nous employons 50 cm³ d'une solution de cystamine à 1%; l'irrigation rectale commence 30 min avant le début de l'irradiation et se poursuit pendant une bonne partie de la durée de celle-ci. Deux agrafes de Michel ferment l'anus pour maintenir en place radium et catheter.

La cytologie rectale a été étudiée sur frottis, de la manière utilisée par nous-mêmes pour la cytologie vaginale et décrite dans nos travaux antérieurs (DAR-CIS¹⁴).

Les prélèvements sont colorés par la méthode de PAPANICOLAOU. Les cellules rectales se présentent sous la forme de cellules ovales, parfois polyédriques ou fusi-formes; leur noyau est ovale ou arrondi, souvent ex-centrique, leur diamètre maximum oscille entre 6,5 μ et 34 μ . Leur protoplasme est basophile, assez homogène, parfois parsemé de fines vacuoles; les limites cellulaires sont nettes; rarement, les cellules renferment deux noyaux. Le plus souvent, les cellules rectales sont iso-lées, il arrive cependant qu'elles soient groupées en amas (Fig. 1).

Tableau I

Pourcentages de cellules rectales radio-lésées dans les jours qui suivent une irradiation intra-rectale pendant 4 h

Jours	Rats													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	—	—	—	—	—	—	4	—	—	3	7	—	—	—
2	—	—	—	—	—	12	—	—	5	—	—	4	5	8
3	10	22	—	—	—	22	19	30	12	—	12	8	10	12
4	—	—	16	29	16	27	18	21	24	22	15	32	20	19
5	6	18	10	33	24	—	20	24	33	40	20	26	15	25
6	12	20	18	24	24	10	7	16	18	12	10	9	25	14
7	21	24	20	24	19	10	5	13	13	4	0	12	18	9
8	24	12	18	22	—	—	8	10	10	0	—	5	8	7
9	—	10	—	—	15	—	5	16	—	5	10	—	0	0
10	—	—	5	18	10	—	—	15	—	—	—	0	—	8
11	—	—	0	6	9	7	—	9	0	—	—	3	5	—
12	10	6	0	—	5	14	0	—	5	—	0	—	—	—
13	—	—	—	—	—	9	—	—	—	—	—	—	—	—

Tableau II

Pourcentages de cellules vaginales radio-lésées dans les jours qui suivent une irradiation intra-rectale pendant 4 h

Jours	Rats														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	2	—	—	4	—	—	4	2	3	C	—	0	—	4	3
2	6	4	—	5	4	5	5	2	4	C	5	C	2	—	5
3	9	—	5	8	C	—	9	C	10	8	6	C	C	5	9
4	13	—	C	18	12	18	—	C	C	13	—	10	17	12	—
5	25	18	C	—	—	19	21	17	C	—	26	21	28	15	37
6	20	22	—	—	25	8	11	8	13	C	—	15	36	29	32
7	13	16	26	21	C	C	—	10	4	22	16	12	27	15	22
8	15	10	19	16	—	5	12	—	—	19	—	9	26	11	16
9	9	—	18	12	7	3	9	11	3	13	—	11	17	8	13
10	8	7	13	14	5	—	8	9	1	—	10	7	12	—	10
11	9	—	8	9	—	—	6	5	2	—	7	5	9	3	8
12	5	—	5	—	—	4	4	—	—	5	—	2	10	2	5
13	—	—	—	—	—	4	2	4	—	—	—	—	—	—	—

Tableau III

Pourcentages de cellules rectales radio-lésées dans les jours qui suivent une irradiation intra-rectale pendant 4 h et après injection intrapéritonéale de cystéamine

Jours	Rats										
	1	2	3	4	5	6	7	8	9	10	11
1	—	5	0	0	—	—	—	—	—	—	—
2	4	8	18	6	—	6	—	—	14	5	0
3	10	6	15	20	10	12	14	6	12	12	12
4	16	14	12	8	21	20	22	26	18	24	6
5	8	4	10	6	10	10	15	6	10	8	4
6	6	3	9	3	7	9	8	4	10	10	—
7	8	8	10	6	—	0	3	0	3	5	0
8	5	5	9	3	5	5	3	—	0	5	2
9	0	6	7	0	—	3	4	—	—	8	0
10	4	5	3	4	—	—	2	—	5	6	—
11	5	0	—	3	0	—	0	—	0	4	—
12	—	—	—	0	—	5	—	—	—	—	—

Après l'irradiation, *apparaissent dans les frottis des cellules radio-lésées*. Nous considérons comme cellule radio-lésée toute cellule nucléée dont le plus grand diamètre dépasse $34\ \mu$ ou qui renferme au moins deux noyaux. La fréquence de semblables cellules dans un frottis normal ne dépasse jamais 7% (Fig. 2 à 5).

Pour chacun des 12 jours qui suivent l'irradiation, nous avons prélevé un frottis vaginal et un frottis rectal et ramené à cent cellules le pourcentage des éléments radio-lésés.

III. Résultats

Nos résultats sont présentés sur les tableaux ci-joints. Ils peuvent être résumés comme suit:

A. Après une irradiation intra-rectale de 4 h sans protection chimique la moyenne des pourcentages maxima de cellules radio-lésées est:

dans le rectum: $26,9 \pm 1,6\%$ (14 rats) (Tableau I);
dans le vagin: $24 \pm 1,7\%$ (15 rats) (Tableau II).

B. Après une irradiation intra-rectale de 4 h, consécutive à l'injection intra-péritonéale de cystéamine (15 mg),

Tableau IV
Pourcentages de cellules vaginales radio-lésées dans les jours qui suivent une irradiation intra-rectale pendant 4 h et après injection intra-péritonéale de cystéamine

Jours	Rats											
	1	2	3	4	5	6	7	8	9	10	11	12
1	0	—	2	—	4	3	3	2	—	—	—	—
2	—	9	—	C	7	5	7	8	0	2	C	0
3	6	7	7	C	9	10	9	14	3	5	C	4
4	9	16	12	C	C	16	10	15	4	7	8	7
5	C	26	15	24	C	14	C	C	8	10	11	8
6	C	20	27	13	14	C	19	20	16	13	17	5 (c)
7	12	14	17	8	6	9	13	14	C	15	9	C
8	5	12	12	6	6	6	9	12	19	11	10	6
9	4	9	8	3	C	5	C	—	18	6	7	5
10	C	5	7	—	1	C	3	6	15	C	4	3
11	1	6	5	3	0	C	2	3	11	4	5	1
12	—	2	1	0	0	2	0	—	7	1	2	3

Tableau V
Pourcentages de cellules rectales altérées dans les jours qui suivent une irradiation intra-rectale de 4 h combinée à une irrigation intra-rectale de cystamine

Jours	Rats										
	1	2	3	4	5	6	7	8	9	10	11
1	—	—	—	—	—	4	5	—	0	5	6
2	—	—	12	4	6	3	10	5	5	15	—
3	12	25	10	12	16	8	8	13	14	4	15
4	15	12	13	5	8	13	12	7	6	9	18
5	3	10	8	8	4	8	20	—	0	—	9
6	4	6	6	—	5	9	7	0	0	0	10
7	0	3	4	5	—	3	4	—	5	0	4
8	0	—	5	6	5	0	0	—	7	3	5
9	—	0	5	2	0	2	0	—	—	—	0
10	3	6	—	0	3	—	4	9	5	—	—
11	—	2	0	—	—	—	6	—	—	0	—
12	6	—	0	0	0	—	—	—	—	—	—

Tableau VI
Pourcentages de cellules vaginales altérées dans les jours qui suivent une irradiation intra-rectale de 4 h combinée à une irrigation intra-rectale de cystamine

Jours	Rats									
	1	2	3	4	5	6	7	8	9	10
1	—	—	1	—	2	4	3	0	C	0
2	2	3	0	2	4 (C)	5	5	—	2	—
3	3	6	6	0	7	9	8	C	4	C
4	C	C	9	C	C	17	9	C	C	5 (C)
5	C	7	—	14 (C)	17	24	C	25	C	10 (C)
6	32	23	16	33	C	30	C	28	27	33
7	27	14	20	25	9	21	24	14	9	28
8	—	9	11	19	C	15	11	9	7	17
9	13	8	7	13	C	8	6	7	C	12
10	4	5	C	10	—	5	4	3	—	9
11	5	6	4	—	—	—	—	4	5	—
12	3	—	1	5	—	—	—	—	2	—

la moyenne des pourcentages maxima de cellules radio-lésées est:

dans le rectum: $19,2 \pm 1,25\%$ (11 rats) (Tableau III);
dans le vagin: $17,2 \pm 1,45\%$ (12 rats) (Tableau IV).

Nous constatons donc une protection à la fois de la muqueuse rectale et de la muqueuse vaginale (radio-protection globale, non spécifique).

C. Après une irradiation intra-rectale de 4 h combinée à une irrigation intra-rectale de cystamine, la moyenne des pourcentages maxima de cellules radio-lésées est:

dans le rectum: $15,8 \pm 1,15\%$ (11 rats) (Tableau V);
dans le vagin: $26,7 \pm 1,80\%$ (10 rats) (Tableau VI).

Il résulte de ces dernières observations que la muqueuse rectale est électivement protégée par la cystamine administrée localement. En effet, si cette protection était due à une résorption de la cystamine dans le torrent circulatoire, le vagin serait lui aussi protégé comme nous l'avons montré en B; ce n'est pas le cas.

Nous pouvons donc conclure que la cystamine est capable d'exercer un effet protecteur local.

IV. Conclusions

Le fait que la cystamine, en application locale, protège le rectum contre une irradiation sans exercer une action d'intensité comparable sur la muqueuse vaginale voisine permet d'envisager pour l'avenir des applications intéressantes, à la radiothérapie des cancers, de certains protecteurs chimiques.

Il est clair que s'il est possible d'accroître la dose-tumeur dans le traitement du cancer du col en évitant, grâce à ces radio-protecteurs introduits sur place, de léser gravement la vessie ou le rectum, un des gros ennuis de la curiethérapie du cancer cervico-utérin sera supprimé ou atténué.

Il ne fait pas de doute pour nous que des techniques de ce genre peuvent être étendues dans d'autres régions du corps, comme le cou, par exemple.

L. DARCIS et P. HOTTERBEECH

Centre anticancéreux près de l'Université de Liège (Belgique), le 7 juin 1957.

Summary

The authors studied the lesions observed in the rectal and vaginal smears of the rat after a local irradiation of the rectum. They observed that an injection of cysteamine before the irradiation strongly diminishes the ratio of damaged rectal and vaginal cells. A local treatment with cysteamine in the rectum before and during the irradiation diminishes only the ratio of damaged rectal cells.

Laboratory Testing of Combined Diphtheria-Tetanus-Pertussis Preparations

Although the efficiency of whooping-cough vaccines has recently been proved in field trials, the problem of combined diphtheria-tetanus-pertussis preparations has been investigated very intensively (UNGAR¹, BOUSFIELD

and HOLT², BARR *et al.*³, SPILLER *et al.*⁴, SAUER and TUCKER⁵, CHEVÉ et ZOURBAS⁶).

Our plan consisted in observing in experimental animals:

- (1) the influence of the whooping cough vaccine on the degree of immunity against diphtheria;
- (2) the influence of the whooping-cough vaccine on the degree of immunity against tetanus;
- (3) the influence of an addition of diphtheria and tetanus antigens and aluminium phosphate on the degree of immunity against whooping-cough.

Preparations employed and methods of testing.—The following preparations were used:

- (1) a combined diphtheria-tetanus purified and adsorbed toxoid (Di-Te);
- (2) a combined diphtheria-tetanus purified and adsorbed toxoid with a pertussis vaccine (Di-Te-Per);
- (3) a whooping-cough vaccine.

The composition of the various components of these preparations was as follows (per ml): Purified diphtheria toxoid 10 Lf, purified tetanus toxoid 10 Lf, 20,000 millions of pertussis germs, 10 mg of aluminium phosphate. Preservative: merthiolate 1:10,000.

To test the amount of immunity against diphtheria and tetanus, the following methods were used: one stimulans method, two stimulans method, toxic challenge method.

Results obtained.—The influence of pertussis germs added to the combined diphtheria-tetanus preparation on the degree of immunity against diphtheria is presented in Table I.

20×10^7 pertussis germs added to 1 ml of combined diphtheria-tetanus preparation had no influence on the degree of immunity against diphtheria, according to the one stimulans method, since there was no significant difference in the average amount of A.U. in the serum of the immunized guinea-pigs. The two stimulans method, however, showed a significant difference in favour of the combined diphtheria-tetanus-pertussis preparation. The results of the toxic challenge method also appeared to be in favour of the combined diphtheria-tetanus-pertussis preparation. According to this method, it appears that an addition of pertussis germs enhances the immunity against diphtheria.

The influence of pertussis germs added to the combined diphtheria-tetanus preparation on the degree of immunity against tetanus is presented in Table II.

20,000 million pertussis germs added to 1 ml of combined diphtheria-tetanus preparation did not influence the degree of immunity against tetanus. A comparison of the degree of immunity against tetanus between the guinea-pigs vaccinated with the combined diphtheria-tetanus preparation, and others vaccinated with the combined diphtheria-tetanus-pertussis preparation, showed no significant difference in the degree of immunity either by the one stimulans method, the two stimulans method or the toxic challenge method.

² G. BOUSFIELD and L. B. HOLT, *Med. Officer* 92, 289 (1954).

³ M. BARR, A. T. GLENNY, and N. R. BUTLER, *Brit. med. J.* 11, 635 (1955).

⁴ V. SPILLER, J. M. BARNES, L. B. HOLT, and D. U. CULLINGTON *Brit. med. J.* 11, 639, 663 (1955).

⁵ L. W. SAUER and W. H. TUCKER, *Amer. J. publ. Health* 44, 784 (1954).

⁶ J. CHEVÉ and J. ZOURBAS, *Atti del II. Congresso Internazionale di Standardizzazione Immunobiologica*, p. 121 (1956).

¹ J. UNGAR, *Proc. Roy. Soc. Med.* 47, 355 (1954).

Table I: Degree of immunity against diphtheria

	One stimulans method		Two stimulans method		Toxic challenge method		
	Di-Te	Di-Te-Per	Di-Te	Di-Te-Per	Dilution	Di-Te Total death	Di-Te-Per Total death
Arithmetic mean of A.U.	3.44	3.064	4.47	6.38	1/100	1/20	1/20
Mean of log. A.U.	0.4856 ± 0.242	0.3714 ± 0.372	0.620 ± 0.101	0.8086 ± 0.104	1/200	19/20	19/20
Geometric mean of A.U.	3.05	2.35	4.17	6.41			
Difference between samples	0.5 > P > 0.4		P < 0.001				

Table II: Degree of immunity against tetanus

	One stimulans method		Two stimulans method		Toxic challenge method		
	Di-Te	Di-Te-Per	Di-Te	Di-Te-Per	Dilution	Total death	Total death
Arithmetic mean of A.U.	2.57	2.66	18.82	17.34	1/50	0/20	0/20
Mean of log. A.U.	0.10 ± 0.661	0.1616 ± 0.869	1.2066 ± 0.169	1.157 ± 0.199	1/100	8/20	8/20
Geometric mean of A.U.	1.251	0.689	16.10	14.30			
Difference between samples	0.5 > P > 0.4		0.5 < P > 0.4				

The pertussis vaccine with addition of aluminium phosphate and diphtheria and tetanus toxoids proved significantly better than the pertussis fluid vaccine ($0.01 > P > 0.001$). In additional experiments, the same vaccine batch to which only aluminium phosphate was added proved also better than the fluid vaccine.

Conclusion.—Laboratory tests of a combined diphtheria-tetanus-pertussis preparation consisting of 30 Lf of purified diphtheria toxoid mixed with 10 Lf of purified tetanus toxoid and 20,000 millions of *H. pertussis* germs, adsorbed on 10 mg of aluminium phosphate, showed that the composition of the preparation was well balanced and that efficient immunity was conferred at the same time against diphtheria, tetanus and whooping-cough.

Acknowledgement.—The author is indebted to J. BENKOVIĆ, LJ. MANDIĆ, N. KÖHLER, and N. ŠKARICA for their help in this work.

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Institute for the Control and Research of Immunobiological Substances, Zagreb (Yugoslavia), July 9, 1957.

Zusammenfassung

In Laboratoriumsuntersuchungen wurde der gegenseitige Einfluss der einzelnen Komponenten des auf Grund purifizierter Anatoxine zubereiteten und auf Aluminiumphosphat adsorbierten Mischimpfstoffes gegen Diphtherie, Tetanus und Pertussis festgestellt.

Pyridoxin and the Acute Toxicity of Isoniazid and Other Acid Hydrazides in Guinea Pigs¹

In an earlier communication from this laboratory², it was demonstrated that the acute toxicity produced in

guinea pigs with acid hydrazides is different from that of free hydrazine compounds. It was further shown that the toxicity of the acid hydrazides alone responded to the administration of pyridoxin. Isoniazid and cyanacetic acid hydrazide were the two substances investigated in that study, and this article represents the results of further experimentation with the hydrazides of pyridine carboxylic, benzoic and acetic acids.

The methods used have been described earlier². A total of 273 guinea pigs were used in this experiment. All compounds, dissolved in water, were administered by intraperitoneal injection. Pyridoxin, when given, was injected immediately before by the same route. The animals were then observed for a period of 20 h for signs of toxicity and the survival time noted.

The results of these experiments show that the central stimulatory action of isoniazid could also be produced by other acid hydrazides whether they are derived from a pyridine carboxylic acid, benzoic acid, or an aliphatic carboxylic acid, such as acetic acid, although the degree of toxicity of these compounds was variable.

From the results presented in Table I, it is seen that pyridoxin exerted a protective effect against the toxicity of all the hydrazides studied. It seems, therefore, that there exists no specific metabolite-antimetabolite relationship between pyridoxin and isoniazid alone. The possibility was considered that pyridoxin simply exerts its effect by forming a hydrazone which may be excreted, as postulated by BIEHL *et al.*³. In order to investigate this problem, ethylidene-isoniazid representing the hydrazone of isoniazid and acetaldehyde, was studied for its toxicity. This substance, it was presumed, cannot chemically interact with pyridoxal as the free end of the hydrazine group is bound. Results indicate that ethylidene-isoniazid was less toxic than isoniazid, and only at higher doses produced symptoms of toxicity, which were modified by pyridoxin. It is possible that ethylidene-isoniazid was hydrolysed within the body to yield free isoniazid, which then produced the toxic effect.

¹ The authors are indebted to J. R. Geigy S.A., Basle, for the preparation of the acid hydrazides studied.

² M. O. TIPUNARAYANAN and W. A. VISCHER, *Exper.* 12, 291 (1956).

³ J. P. BIEHL and R. W. VILTER, *Proc. Soc. exp. Biol. Med.* 85, 389 (1954).

Table I
Effect of Pyridoxin on the Acute Toxicity of Acid Hydrazides in Guinea Pigs

mg/kg	Minimal mean survival time, in hours									
	Iso-nicotinic acid hydrazide		Picolinic acid hydrazide		Nicotinic acid hydrazide		Benzoic acid hydrazide		Acetic acid hydrazide	
	A	B	A	B	A	B	A	B	A	B
100	> 20 (5)	—	> 20 (4)	—	—	—	> 20 (3)	—	—	—
150	—	—	15 (4)	20 (3)	—	—	7 (6)	20 (3)	20 (3)	—
175	—	—	4.5 (3)	20 (3)	—	—	1.5 (3)	20 (3)	1.5 (2)	—
200	> 20 (5)	> 20 (5)	7.5 (3)	16.6 (3)	—	—	1.3 (3)	16 (6)	1.5 (2)	> 20 (2)
250	—	—	1 (5)	17 (3)	—	—	1.5 (3)	1 (3)	1 (3)	20 (1)
300	2.5 (5)	> 20 (5)	1.25 (1)	2.6 (3)	2.3 (3)	> 20 (4)	1 (3)	1 (3)	1 (3)	20 (2)
400	2 (5)	> 20 (5)	1.25 (2)	0.75 (3)	1.5 (3)	2.3 (3)	1 (3)	1 (2)	1 (2)	19 (11)
500	1.75 (5)	3 (5)	1.1 (2)	1.6 (3)	1.3 (3)	1.5 (3)	1 (2)	—	—	13 (10)
600	—	—	—	—	0.75 (3)	1.25 (3)	—	—	—	13 (10)
750	—	—	—	—	—	—	—	—	—	—

A = no vitamin; B = with pyridoxin, 300 mg/kg.

Figures in () indicate the number of animals used.

Table II
In vitro Tuberculostatic Activity of Acid Hydrazides

	Minimal inhibitory concentration*, γ/ml	
	H37Rv	H37Rv isoniazide-resistant
Isoniazid	0.1	> 100
Nicotinic acid hydrazide	> 100	> 100
Picolinic acid hydrazide	10	> 100
Benzoic acid hydrazide	> 100	> 100
Acetic acid hydrazide	> 100	> 100
Ethylidene-isoniazid	0.1	> 100
iso-Nicotinic acid	> 100	> 100
iso-Nicotinamide	> 100	> 100

* Minimal inhibitory concentration determined in Youmans medium after three weeks incubation at 37°C

Results presented in Table II show that, among the substances tested, only picolinic acid hydrazide and ethylidene-isoniazid had antituberculous activity comparable to that of isoniazid, they were also ineffective against isoniazid-resistant tubercle bacilli. From the results of these experiments, it can be concluded that the acute toxicity of isoniazid is not specific as it is produced by other acid hydrazides; the acute toxicity of all acid hydrazides studied is modified by pyridoxin; ethylidene-isoniazid, as an isoniazid-hydrazone, has diminished toxicity comparable to a combination of pyridoxin and isoniazid; and the anti-tuberculous activity of the acid hydrazides and their toxic effects are produced by two different mechanisms.

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Zusammenfassung

Die Autoren konnten nachweisen, dass Isoniazid und Säurehydrazide die gleichen toxischen Symptome verursachen. Pyridoxin zeigt eine schützende Wirkung gegen die Toxizität aller Säurehydrazide. Ferner konnte nachgewiesen werden, dass die antituberkulöse Aktivität der Säurehydrazide und ihre Toxizität verschiedenen Wirkungsmechanismen angehören.

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Opsonic Activity of Properdin

It has been recently suggested that properdin, a protein present in the serum of normal mammals, is involved in the mechanism of the natural immunity against infections (LANDY and PILLEMER)¹. In fact, this substance possesses a marked bactericidal action on Gram-negative bacteria and is able to produce also complete inactivation of several types of pathogenic viruses. Modifications of the properdin content of the sera have been reported to occur in several pathological conditions (FRANK *et al.*²,

¹ M. LANDY and L. PILLEMER, J. exp. Med. 104, 383 (1956).
² E. FRANK, J. FINE, and L. PILLEMER, Proc. Soc. exp. Biol., N.Y. 89, 223 (1955).

Influence of different treatments and additions on the opsonic power of guinea pig serum

Additions	Normal serum	Serum inactivated 56°C	Serum + Zymosan	No serum
None	7.16 $\pm$ 1.65	2.75 $\pm$ 0.46	2.73 $\pm$ 0.40	—
Properdin in barbital buffer . . .	9.21 $\pm$ 2.12	7.83 $\pm$ 1.75	8.63 $\pm$ 2.41	1.36 $\pm$ 0.29
Properdin in barbital buffer + Mg ⁺⁺	13.23 $\pm$ 1.36	13.74 $\pm$ 0.92	13.46 $\pm$ 0.89	2.91 $\pm$ 0.66
Barbital buffer	6.09 $\pm$ 1.83	—	—	2.66 $\pm$ 0.50
Barbital buffer + Mg ⁺⁺	12.59 $\pm$ 1.54	—	—	2.82 $\pm$ 0.64
Physiological saline	—	—	—	2.32 $\pm$ 0.13
No. of experiments	6	6	6	6

The values show the average number of *Staphylococci* phagocytized by one granulocyte $\pm$ standard deviation.

HEGEMANN³, LANDY and PILLEMER⁴) and particularly in animals treated with the somatic antigens (lipopolysaccharides) of Gram-negative bacteria. The correspondence of an increase of properdin blood levels with an enhancement of the natural resistance to the infections has been emphasized (LANDY and PILLEMER).

Properdin does not exert bactericidal action on Gram-positive microorganisms such as *Staphylococcus pyogenes* or *Mycobacterium tuberculosis* (LANDY and PILLEMER), but protection of animals determined by the treatment with lipopolysaccharides of Gram-negative bacteria exists also in the case of *Staphylococcus pyogenes* or of *Mycobacterium tuberculosis* infections (DUBOS *et al.*⁵). It seems then evident that the bactericidal power cannot entirely explain the mechanism of action of properdin.

The properdin system which exerts full bactericidal effect consists of properdin, complement and magnesium ions. It is inactivated by zymosan and by heating at 55–57°C.

The presence in normal sera of a substance (or substances) capable of preparing the bacteria for the phagocytosis by granulocytes was firstly described by SANARELLI (1891), but the first complete description was given by WRIGHT (1904). This author called these substances 'opsonins'.

Opsonin has been described as composed of a thermolabile as well as a thermostable portion⁶. The thermolabile fraction has been thought to be identical with complement (VERNONI)⁷.

The possible relationship between the thermolabile fraction of opsonin and properdin is discussed in this paper.

Normal guinea pigs weighing 300–400 g used in this investigation provided the source of serum. The blood was taken directly from the heart of 18-h-fasted animals and the serum was used immediately after separation. Leucocytes were obtained from the peritoneal cavity of other guinea pigs, in which 10 ml of cultural broth were injected; 6 h after this injection, the fluid present in the peritoneal cavity was extracted by means of a Pasteur pipette and centrifuged at 1000 g for 20 min. The sedimented leucocytes were washed 3 times and then suspended in 5 ml of physiological saline. Bacterial suspensions were prepared from 24 h broth cultures of *Staphylococcus pyogenes aureus* var. Oxford. They contained about

2 billions living microorganisms in 7 ml of physiological saline. For the measurement of the opsonic capacity of the serum, 0.5 ml of the leucocytes suspension was mixed with 0.5 ml of the serum and with 0.5 ml of the bacterial suspension. The mixture was allowed to stand at 37°C for 20 min, and then centrifuged at 1000 g $\times$ 15 min. The sediment was washed once and then stripped on a glass slide. The specimen was then stained by the method of GRAM modified by NICOLLE. The number of cocci phagocytized by an individual leucocyte was counted under a microscope. At least 100 leucocytes were counted each time. The opsonic capacity was expressed as 'opsonic power', i.e. the number of cocci counted within 100 granulocytes divided by 100.

Properdin was prepared from human serum according to the method of PILLEMER⁸. It was stored at –18°C. The amount used in each experiment corresponded to 5 ml of fresh serum; it was dissolved in barbital-acetate buffer, pH 7.4.

Zymosan was prepared from baker's yeast according to the method of PILLEMER⁹. When the serum was treated with zymosan in order to block properdin 4 mg of zymosan were used for 1 ml serum. Incubation lasted for 75 min at a temperature of 17°C. Magnesium ions, when added, had the concentration 0.0012 M. The results are summarized in the Table. It seems clear from this table that the treatment of normal serum with zymosan produces a marked decrease of the 'opsonic power'; the degree of this decrease resembles that determined by heating at 56°C for 30 min. Addition of properdin to the serum inactivated by heating or by treatment with zymosan restores completely the normal values. Addition of properdin to fresh serum does not increase the opsonic power. Addition of magnesium always brings about a strong increase. Neither properdin nor magnesium exerted any effect when used alone in the absence of serum. These results confirm that opsonin consists of 2 fractions, the first of which is thermolabile while the second one is not destroyed at 56°C. Since the thermolabile fraction is destroyed also by zymosan, and its full activity can be restored by the addition of properdin, it seems highly probable that the thermolabile fraction of opsonin is identical to properdin.

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Institute of General Pathology, University of Genoa (Italy), July 9, 1957.

³ F. HEGEMANN, Z. Immunit. Forsch. 3, 202 (1954).

⁴ M. LANDY and L. PILLEMER, J. exp. Med. 103, 823 (1956).

⁵ R. J. DUBOS and R. W. SCHAEGLER, J. exp. Med. 104, 53 (1956).

⁶ S. MUDD, B. LUCKÉ, M. McCUTCHEON, and M. STRUMIA, J. exp. Med. 49, 779 (1929).

⁷ G. VERNONI, Patologia Generale (Ed. Sansoni, 1954), p. 540.

⁸ L. PILLEMER, L. BLUM, I. H. LEPOW, O. A. ROSS, E. W. TODD, and A. C. WARDLAW, Science 120, 279 (1954).

⁹ L. PILLEMER, L. BLUM, J. PENSCKY, and I. H. LEPOW, J. Immunol. 71, 331 (1953).

Riassunto

L'aggiunta di zimosano al siero normale di cavia ne diminuisce notevolmente il potere opsonico; l'aggiunta di properdina al siero inattivato con zimosano o col calore restaura interamente questo potere. La properdina usata da sola senza siero, non ha potere opsonico. Sembra molto probabile che la frazione termolabile delle opsonine sia da identificare con la properdina.

DDT-Analogs as Synergists for DDT

Diaryl-trifluoromethyl-carbinols $\text{Ar}_2\text{C}(\text{OH}) \cdot \text{CF}_3$ have been found to be active as synergists against a strain of moderately resistant houseflies¹; the most active compound of the series was the di-(*p*-chloro)-derivative. It seemed of interest to investigate analogs containing chlorine and/or fluorine in the methyl group and also to study the importance of the hydroxyl group present in these compounds.

The compounds tested have been described by BERGMANN *et al.*², apart from di-(*p*-chlorophenyl)-dichloromethyl-carbinol and 1,1-di-(*p*-chlorophenyl)-2,2-dichloroethane which have been reported by PEPPER and KULKA³ and by HALLER *et al.*⁴, respectively. The housefly species used in these experiments were a susceptible strain ('T') and a 200 times more resistant strain ('R') of *Musca vicina*. Benzene solutions of the chemicals were applied *topically* to groups of 25 flies. Mortality counts were made after 24 and 48 h. Each compound was tested in at least four concentrations with 5 to 8 repetitions. The experimental procedure has been described in detail by TAHORI¹.

The Table summarises the results obtained; it indicates the insecticidal activity of each compound as well

as its synergist value (for a ratio of DDT: synergist = 10:1). In order to characterize the slope of the dosage-mortality curves, the LD₁₅ and LD₈₅ values are given in parenthesis.

Against *susceptible* flies, none of the carbinols approaches DDT in insecticidal activity. It is noteworthy that all those diarylmethyl-carbinols in which the methyl group is fully substituted (CF_3 , CF_2Cl , CFCl_2) (VI–VIII) are fairly active on topical application, though on tarsal application no effect has been found for di-(*p*-chlorophenyl)-trifluoromethyl-carbinol⁵. It can be predicted that the same will probably hold true for di-(*p*-chlorophenyl)-trichloromethyl-carbinol. This carbinol and the dichloromethyl analog (IX) have been reported by GUNTHER, BLINN, and METCALF⁶ to be of low toxicity to *Musca*. The di-(*p*-chlorophenyl)-trichloromethyl carbinol used by these authors, of Rohm and Haas manufacture, appears to be different from the compound used by REUTER and ASCHER, and to which the same formula had been assigned. On the other hand, the diaryl-dihalomethyl-carbinols (IX–XIII) exhibit lower activity; this activity rises from the difluoro- over the fluoro-chloro- to the dichloromethyl compound. One is tempted to assume that, as in the case of DDT, the size of the substituents of the methyl group is the decisive factor, causing a distortion of the tetrahedron of the arylated carbon atom into a trihedral configuration⁷; chlorine is effective, fluorine too small. Another observation may be of interest in this connection. The inactive carbinols are easily dehydrated, the active ones not: the trihalogenomethyl compounds can, of course, not be dehydrated at all, but we have noted that also di-(*p*-chlorophenyl)-dichloromethyl-carbinol offers a strong resistance to every attempt at dehydration.

All carbinols tested are more active insecticides than DDT for *resistant* houseflies; again the two groups defined above are clearly distinguished.

¹ A. S. TAHORI, J. econ. Ent. 48, 638 (1955).

² E. D. BERGMANN, P. MOSES, M. NEEMAN, S. COHEN, A. KALUSZYNER, and S. REUTER, J. Amer. chem. Soc. 79, 4174 (1957).

³ J. M. PEPPER and M. KULKA, J. Amer. chem. Soc. 72, 1417 (1950).

⁴ HALLER *et al.*, J. Amer. chem. Soc. 67, 1596, 1600 (1945).

⁵ S. REUTER and K. R. S. ASCHER, Exper. 12, 316 (1956).

⁶ F. A. GUNTHER, R. C. BLINN, and R. L. METCALF, J. Food Agr. Chem. 4, 338 (1956).

⁷ E. F. ROGERS, H. D. BROWN, I. M. ROSSMUSSEN, and R. E. HEAL, J. Amer. chem. Soc. 75, 2991 (1953).

	Compound	Insecticidal Activity in µg/fly		Toxicity in µg of DDT/fly + compound at a ratio of 10:1	
		R	T	R	T
I	(<i>p</i> -ClC ₆ H ₄) ₂ ·CH·CCl ₃	140 (80; 250)	0·7 (0·4; 1·1)	140 (80; 250)	0·7 (0·4; 1·1)
II	(<i>p</i> -ClC ₆ H ₄) ₂ ·CH·CF ₃	13 (8; 21)	4 (2; 7)	13 (8; 22)	0·2 (0·1; 0·4)
III	(<i>p</i> -ClC ₆ H ₄) ₂ ·CH·CF ₂ Cl	50 (32; 77)	14 (9; 19)	14 (8; 23)	0·4 (0·2; 0·8)
IV	(<i>p</i> -ClC ₆ H ₄) ₂ ·CH·CFCl ₂	115 (68; 190)	5 (3; 8)	30 (17; 51)	0·4 (0·2; 0·7)
V	(<i>p</i> -ClC ₆ H ₄) ₂ ·CH·CHCl ₂	> 140	4 (2; 7)	140	0·6 (0·3; 1·0)
VI	(<i>p</i> -ClC ₆ H ₄) ₂ ·C(OH)·CF ₃	6 (3; 11)	5 (3; 7)	9 (5; 18)	0·3 (0·2; 0·5)
VII	(<i>p</i> -ClC ₆ H ₄) ₂ ·C(OH)·CFCl ₂	5 (3; 9)	6 (4; 11)	13 (7; 21)	0·5 (0·3; 1·0)
VIII	(<i>p</i> -ClC ₆ H ₄) ₂ ·C(OH)·CFCl ₂	5 (3; 9)	6 (4; 11)	13 (7; 21)	0·5 (0·3; 1·0)
IX	(<i>p</i> -ClC ₆ H ₄) ₂ ·C(OH)·CHCl ₂	4 (2; 7)	1 (0·5; 2)	15 (9; 27)	0·2 (0·1; 0·4)
X	(<i>p</i> -ClC ₆ H ₄) ₂ ·C(OH)·CHFCl	11 (7; 18)	9 (5; 14)	13 (7; 21)	0·4 (0·3; 0·7)
XI	(C ₆ H ₅) ₂ ·C(OH)·CHF ₂	70 (43; 110)	52 (37; 72)	66 (38; 115)	0·4 (0·2; 0·7)
XII	(<i>p</i> -ClC ₆ H ₄) ₂ ·C(OH)·CHF ₂	27 (16; 46)	31 (18; 56)	27 (16; 46)	0·4 (0·2; 0·7)
XIII	(<i>p</i> -BrC ₆ H ₄) ₂ ·C(OH)·CHF ₂	31 (19; 49)	27 (17; 45)	31 (20; 48)	0·3 (0·2; 0·7)
XIV	(<i>p</i> -ClC ₆ H ₄) ₂ ·C(OOCCH ₃)·CF ₃	7 (5; 13)	4 (3; 7)	9 (4; 18)	0·2 (0·1; 0·4)
XV	(<i>p</i> -ClC ₆ H ₄) ₂ ·C(OOCCH ₃)·CF ₂ Cl	80 (47; 130)	150 (150)	36 (18; 70)	0·4 (0·2; 0·7)
XVI	(<i>p</i> -ClC ₆ H ₄) ₂ ·C(OOCCH ₃)·CFCl ₂	> 200	> 200	39 (15; 100)	0·5 (0·3; 0·8)

Toxicities of some DDT analogs to 'R' and 'T' strains of *M. vicina* (The values in brackets indicate LD₁₅ and LD₈₅)

A similar picture is obtained from the figures dealing with the *synergist* activities of the carbinols. In the case of *susceptible* flies, their effect is small (lowering the value of 0.7 for DDT to 0.3–0.4), whilst for the *resistant* strain the 'active' carbinols increase the toxicity of DDT about 10 times, the 'inactive' ones much less. The distinction between the two groups is clear only in the latter case.

Replacement of the hydroxyl group in the carbinols by hydrogen (compounds II–V) produces compounds which are less active than DDT in susceptible and somewhat more active than the standard compound in resistant flies. If in the latter case the differences are significant, it appears that the sequence of the activities is the reverse from what one would have expected on the basis of the 'trihedral theory': $\text{CF}_3 > \text{CF}_2\text{Cl} > \text{CFCl}_2$, whilst the size of the halogenated methyl group varies accordingly to $\text{CFCl}_2 > \text{CF}_2\text{Cl} > \text{CF}_3$. This is true both for the insecticidal power and the synergist activity of the compounds.

Acetylation of the hydroxyl group in the 'active' carbinols (XIV–XVI) destroys the activity for the fluorodichloro and—to a lesser degree—for the difluorochloro compound. The trifluoro compound (XIV) has the same effect as the free carbinol. It can be assumed that in the latter case the esterases of the body are capable of hydrolyzing the acetyl compounds. Indeed, XIV reaches its activity only after 72 h. With increasing size of the methyl substituents, the hydrolysis becomes more difficult—perhaps as a result of steric hindrance. One would then expect that the acetate of di-(*p*-chlorophenyl)-trichloromethyl-carbinol would be completely inactive.

The mode of action of the 'active' carbinols is not quite clear. They may function as unspecific narcotics, as proposed by GAVAUDAN and POUSSEL⁸ for DDT. In this respect, it may be recalled that the vapour of di-(*p*-chlorophenyl)-trifluoromethyl-carbinol has a certain depressant effect on flies⁹. Experiments in this direction are now in progress.

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The Medical Research Laboratories, Army Medical Corps, Israel Defence Forces, June 25, 1957.

Résumé

L'influence de la halogénuration du groupement méthyl dans le di-(*p*-chlorophényl)-méthyl-carbinol sur l'activité de ce composé comme insecticide ou synergiste du DDT a été étudiée. On essaye de donner une explication rationnelle des faits observés.

⁸ P. GAVAUDAN and H. POUSSEL, C. r. Acad. Sci. 224, 683 (1947).

⁹ S. COHEN and A. S. TAHORI, J. Agr. Food Chem. 5, 519 (1957).

The Enzymatic Degradation of Yeast Nucleic Acid by Normal Rat Liver Tissue¹

Using rat liver homogenates and mitochondrial preparations respectively, DELAMIRANDE *et al.*² and ROTH³

¹ This work was supported by a grant (C-2459C2) from the U. S. Department of Public Health.

² G. DELAMIRANDE, C. ALLARD, H. C. DA COSTA, and A. CANTERO, Science 119, 351 (1954).

³ J. S. ROTH, J. biol. Chem. 208, 181 (1954).

found two optima in measuring the pH activity of their preparations against yeast nucleic acids. On the basis of these findings they postulated the presence of an acid as well as of alkaline ribonuclease in normal rat liver tissue. MAVER and GRECO⁴ reported similar results. According to ROTH⁵ the alkaline ribonuclease is similar or identical with the crystalline pancreatic ribonuclease, whereas the peak on the acid side is due to the action of a non-specific diesterase.

Table I
Enzymatic Hydrolysis of Yeast Nucleic Acid by Rat Liver Ribonuclease Plus Prostate Acid Phosphatase

pH	% I.P. Based on the total P of substrate
5.2–8.9	93–100%

Applying to normal liver homogenates the same method of extraction⁶ which yielded a soluble enzyme preparation in the case of the cells of the mouse ascites tumor, we confirmed the presence of two peaks in the pH activity curve using 0.14 veronal buffer (1–2 cm³ rat liver extract corresponding to 1–2 g rat liver; 10 mg yeast nucleic acid: Total volume 10 cm³). Maximal depolymerization as measured by determination of the acid soluble P, after precipitation with MacFadyen's reagent, was observed at pH 5.6 and 8.6 respectively after 6–8 h at 37° C. Moreover the rate of hydrolysis at these pH optima was found to be approximately the same. After exhaustive digestion (40–48 h) all the diester bonds of yeast nucleic acid were found to be cleaved, as suggested by the amount of I. P. after 3 h incubation with purified prostate acid phosphatase at pH 5.6 (Table I). Therefore, it does not seem that this 'alkaline' ribonuclease can be identical with or even similar to pancreatic ribonuclease, since pancreatic ribonuclease is known to hydrolyze linkages in yeast nucleic acid involving pyrimidine nucleotides only, and not all diester bonds. Moreover, purine polynucleotides (prepared by exhaustive digestion of yeast nucleic acid with crystalline ribonuclease) were also found to be cleaved completely by this preparation, and the pH activity curve showed the same two optima at pH 5.6 and 8.6 respectively.

In connection with the above results the observation of HIRS *et al.*⁷ are of interest. Their chromatographic analyses of acid extracts of beef liver did not reveal the presence of either of the two enzymes of the pancreas with ribonuclease activity.

Our preparations apparently contain only ribonuclease and no non-specific diesterase activity since glycerolphosphorylcholine was not cleaved at any pH.

Still, the two peaks in the pH activity curve seem to be due to the presence of two ribonucleases. Treatment of the enzyme preparation with sulfuric acid (standing in 0.25 M sulfuric acid for 24 h, at 5° C) revealed a difference in its hydrolytic behaviour at pH 5.6 and 8.6 respectively (Table II). There was no appreciable cleavage at pH 8.6. The partial inactivation at pH 5.6 involved reduction to about the same degree of the

⁴ M. E. MAVER and A. E. GRECO, J. nat. Cancer Inst. 17, 503 (1956).

⁵ J. S. ROTH, Fed. Proc. 15, 341 (1956).

⁶ F. STECKERL, Arch. Biochem. Biophys. 58, 73 (1956).

⁷ C. H. W. HIRS, W. H. STEIN, and ST. MOORE, Congr. int. Biochem. Résumés communications 2e Congr. (Paris 1952), p. 258.

Table II
Enzymatic Hydrolysis of Yeast Nucleic Acids by the H₂SO₄-treated Enzyme at pH 5.6 (A); at pH 8.6 (B); by Crystalline Ribonuclease (C); by A + C. Acid Phosphatase Added to all Digestion Mixtures for 3 h (pH 5.6).

	% I.P. based on the total P of substrate
A	50–55%
B	8–10%
C	50%
A + C	72–75%

hydrolytic activity towards either bonds, those involving purine as well as pyrimidine nucleotides. The *I. P.* in excess of 50% in the experiments in which crystalline ribonuclease plus partially inactivated liver ribonuclease were used must come from purine nucleotides. Therefore the other 25–28% of *I. P.* in the experiments in which only the partially inactivated enzyme was used, must come from pyrimidine nucleotides.

As final products of hydrolysis, using yeast nucleic acids as well as cyclic nucleotides as substrates, we found purine and pyrimidine mononucleotides esterified at the 2 and 3 position of the ribose moiety, their relative proportion varying from 40–60% at either pH. (The acid monoesterase was inhibited by 0.03 *M* fluoride, the alkaline by 0.05 *M* arsenate.) We employed COHN's method⁸ for their identification. No isomerase activity could be detected in our preparations.

The two kinds of split products suggested the presence of two different enzymatic activities in our preparations.

Following the procedure of DAVIS and ALLEN⁹ we absorbed the enzyme preparation on IRC-50 which had been equilibrated with 0.1 *M* acetate buffer at pH 6.0. The concentrated filtrate (1 cm³ = 1 g of liver tissue) cleaved nucleic acid only to a slight degree (about 5 to 10%); the cyclic nucleotides were found to be split to 2 nucleotides to about 85%, the remaining 15% being 3' nucleotides. Activity was about the same at pH 5.6 and 8.6.

The column was then washed with 1 *M* acetate buffer at pH 6.0, and the concentrated eluate was found to cleave yeast nucleic acid to 3' nucleotides to the extent of 90%, the remaining 10% being 2' nucleotides at pH 5.6. The same hydrolysis products were obtained with cyclic nucleotides as substrates. At alkaline pH no appreciable activity was observed.

It might well be that the alkaline ribonuclease was inactivated by this procedure; on the other hand one should recall the finding of WILLSTÄTTER¹⁰ that impure gastric lipase acted best at pH 4–5, while after purification optimal action was observed at pH 8. Apparently removal or changes of different contaminating proteins may markedly influence the pH activity curve of an enzyme.

F. STECKERL, JOANN KING,
and A. OFODILE

Department of Biochemistry, Boston University School of Medicine, Boston (Mass.), June 18, 1957.

⁸ W. E. COHN and E. VOLKIN, *Nature* **167**, 483 (1951).
⁹ F. F. DAVIS and F. W. ALLEN, *Biochim. biophys. Acta* **21**, 14 (1956).
¹⁰ R. WILLSTÄTTER, E. WALDSCHMIDT-LEITZ, and F. MEMMEN, *J. physiol. Chem.* **125**, 93 (1923).

Zusammenfassung

Die Befunde zeigen, dass am Abbau von Hefenukleinsäure durch Rattenleberextrakte zwei Fermente wirksam sind: a) eine Ribonuklease, welche 3-Nukleotide als Spaltprodukte liefert, und b) eine Phosphodiesterase, welche zyklische Nukleotide zu 2-Nukleotiden abbaut.

The Dose-Response Regression Lines of Pertussis Vaccines with and without Aluminium Phosphate

We tested a combined diphtheria-tetanus-pertussis vaccine adsorbed on aluminium phosphate, a pertussis monovaccine adsorbed on aluminium phosphate, and a pertussis monovaccine without addition of aluminium phosphate. The same batch of pertussis vaccine was used in both monovaccines and in the combined diphtheria-tetanus-pertussis vaccine. In our experiments, we made use of the active mouse-protection test and examined the dose-response regression lines of the fluid and adsorbed pertussis vaccines.

Since each vaccine series used in our experiments contained the same amount of active substance of the *H. pertussis* germ, it might have been expected that there would be no significant departure from parallelism of the dose-response regression lines, and that the degree of protection afforded by all three series would be almost the same.

As one of our vaccine series contained aluminium phosphate and the other had been prepared without aluminium phosphate, we were in a position to observe the influence of aluminium phosphate on the dose-response regression lines. On the other hand, as one of the vaccine series contained both aluminium phosphate and diphtheria and tetanus anatoxins, and the other only aluminium phosphate, we had the opportunity of observing the influence of diphtheria and tetanus anatoxins on the dose-response regression lines as well.

Results.—Since an international standard vaccine against whooping-cough is to be adopted, and the value of the various vaccine series is to be expressed in terms of the international standard, the influence of aluminium phosphate not only on the degree of immunity against whooping-cough but also on the dose-response regression lines of vaccines, with and without aluminium phosphate, is worthwhile observing.

An addition of aluminium phosphate changes the dose-response regression lines of diphtheria toxoid in such a way that they are not parallel with the dose-response regression lines of a prophylactic without aluminium phosphate (HOLT¹). The same occurs with an addition of aluminium hydroxide (JERNE and WOOD²).

The following table shows a statistical analysis of the departure from parallelism of the dose-response regression lines of all the three vaccines tested, compared with the reference (pertussis fluid) vaccine prepared at our Institute. The tests were repeated six times.

The statistical analysis of the departure from parallelism of the dose-response regression lines between the pertussis fluid vaccine and the reference fluid vaccine showed no significant difference, but there was a signifi-

¹ L. B. HOLT, *First European Meeting of Biological Standardisation—Diphtheria Toxoid* (Lyon 1955).
² N. K. JERNE and E. C. WOOD, *Biometrics*, American Statistical Association **5**, 273 (1949).

Departure from parallelism	Di-Te-Per adsorbed	Pertussis adsorbed	Pertussis fluid
$S_1-S_2-T_1+T_2$	$0.05 > P > 0.02$	$0.02 > P > 0.01$	$0.4 > P > 0.3$

cant difference between the pertussis or Di-Te-Per vaccines with addition of aluminium phosphate and the fluid vaccines. Thus our hypothesis that we were assaying two samples of the same active substance was contradicted by the results.

Should further experiments show that an addition of aluminium phosphate to pertussis vaccines might influence the degree of immunity against whooping-cough, and that in such a case aluminium phosphate does not behave as an inert substance but influences the dose-response regression lines, difficulties might arise in expressing the value of a vaccine containing aluminium phosphate in terms of an international standard that would not contain aluminium phosphate. The difference in protection between the same series of a pertussis vaccine with and without addition of aluminium phosphate would depend on the dose used for comparing the vaccine. In our case, the difference was very slight with a larger dose, whereas with a smaller dose it proved to be three times in favour of the vaccine with an addition of aluminium phosphate. An addition of diphtheria and tetanus toxoids to a pertussis vaccine did not influence the dose-response regression lines.

Acknowledgment. – The author is indebted to J. BENKOVIĆ, LJ. MANDIĆ, N. KÖHLER, and N. ŠKARIĆ for their help in this work.

D. IKIĆ

Institute for the Control and Research of Immunobiological Substances, Zagreb (Yugoslavia), July 9, 1957.

Zusammenfassung

In Laboratoriumsuntersuchungen wurde der Einfluss von Aluminiumphosphat auf die «dose-response regression lines» des Impfstoffes gegen Keuchhusten untersucht.

Multiple Dietary Necrotic Degeneration in the Mouse

A fatal deficiency disease, multiple necrotic degeneration, can be produced in mice by feeding a semi-synthetic diet which is based on dried American *Torula* yeast as the sole source of protein¹. The ration which has been used for the production of liver necrosis in rats², is low in cystine, and simultaneously deficient in two essential dietary factors, namely, vitamin E, and Factor 3³. The pathology of multiple necrotic degeneration in the mouse is characterized by extensive necrotic changes in the heart muscle, liver, kidneys, and peripheral muscle (Fig. 1). The massive necrosis of the heart predominates.

¹ General composition: Sucrose 59, *Torula* yeast 30, vitamin E-free lard 5, salts 5, and a vitamin mixture (in lactose) 1. Vitamin A acetate (1 mg%) and vitamin D₂ (1 µg%) are added.

² K. SCHWARZ, Proc. Soc. exp. Biol. Med. 77, 818 (1951).

³ K. SCHWARZ, Ann. N. Y. Acad. Sci. 57, 878 (1954). – The term 'Factor III' has more recently been used for a form of vitamin B₁₂ [W. FRIEDRICH and K. BERNHAUER, Angew. Chem. 65, 627 (1953)]. There is no relation between Factor 3 against necrotic liver degeneration and the vitamin B₁₂-Factor III.

The changes are associated with a pronounced pancreatic atrophy and with degeneration of the testes. In the present studies, weanling, male F1 progeny from the cross between female BALB/cAnN and male DBA/2JN strains of homozygous mice was used⁴. The mice, weighing between 8 and 15 g at weaning, were kept in individual wire-mesh bottom cages at a constant temperature of 23° to 25°C. Diets were fed *ad libitum*. Supplements (Factor 3, vitamin E, or cystine) were mixed into the dry basal ration. Control mice were maintained on a semi-purified diet containing 30% casein.

The outcome of five successive experiments (A–E) is presented in the Table. Death occurred on the basal diet between the 38th and 96th day. The average survival times of these groups ranged from 65 to 73 days. The overall incidence of necrotic lesions in different organs was: Heart 91, liver 54, muscle 47, and kidneys 42%. Multiple necrotic degeneration appears to develop in stages: Between the 38th and the 50th day necrotic lesions were found only in the heart. The organ was frequently enlarged. The valves and the endocardium were normal. After 50 days liver necrosis was seen as well. This lesion was indistinguishable in appearance from dietary liver necrosis in the rat, but it did not precipitate the acute, terminal breakdown seen in the latter species. The majority of mice surviving for 68 or more days also had skeletal muscle degeneration (muscular dystrophy), kidney lesions, and atrophy of the pancreas. The kidneys were enlarged and showed white spots uniformly distributed over their surface. Occasionally hematuria was noted. The pancreatic atrophy was restricted to the secretory portion of the gland (Fig. 2). The picture was reminiscent of the acinar atrophy observed in kwashiorkor⁵. In the latter stages of multiple necrotic degeneration there was also atrophy of the testes.

Either Factor 3 or vitamin E alone prevented multiple necrotic degeneration in the mouse (Table). Cystine had only a partial effect⁶. The protective effects are in good correlation with those obtained against liver necrosis in the rat. The Factor 3 concentrate used in experiment D was prepared by fractionation from brewers yeast. It prevented the gross pathological changes for an extended time⁷. The observed protective effects were paralleled by significant differences between the growth rates of the various groups.

Factor 3, described as an independent dietary agent in 1951 by SCHWARZ⁸, is water soluble, strongly bound to protein and organic in nature. It has been obtained from various natural sources in high concentration⁹. Recently, Factor 3 has been shown to contain selenium in organically bound form¹⁰. It is extremely potent for preventing liver necrosis in the rat, and exudative diathesis in the chick¹¹. Factor 3 can be replaced by

⁴ The cooperation of the Animal Production Section, National Institutes of Health, is gratefully acknowledged.

⁵ H. C. TROWELL, J. N. P. DAVIES, and R. F. A. DEAN, *Kwashiorkor* (Edward Arnold Ltd., London 1954).

⁶ The effect of L-cystine has been found to be caused by a trace contamination with selenium. K. SCHWARZ, unpublished results.

⁷ With the exception of the testicular atrophy.

⁸ K. SCHWARZ, Proc. Soc. exp. Biol. Med. 78, 852 (1951).

⁹ K. SCHWARZ *et al.*, unpublished results.

¹⁰ K. SCHWARZ and C. M. FOLTZ, J. Amer. chem. Soc. 79, 3292 (1957).

¹¹ K. SCHWARZ, J. G. BIERI, G. M. BRIGGS, and M. L. SCOTT, Proc. Soc. exp. Biol. Med. 95, 621 (1957).

Multiple Necrotic Degeneration in the Mouse (Summary of five experiments)

Diet	Ex- periment	Average weight gain in 8 weeks (in g)	Duration (in days)	Number of		Incidence of necrotic degeneration in			
				Animals	Deaths	Heart	Liver	Muscle	Kidney
Control (casein diet)	A	15.2	200	21	0	0	0	0	0
	B	14.5	140	15	0	0	0	0	0
	C	14.2	79	5	0	0	0	0	0
	Totals	14.7	—	41	0	0	0	0	0
Basal (<i>Torula</i> yeast diet)	A ¹	6.2	77	15	10 ²	— ³	10	6	10
	B	8.2	96	16	11 ²	15	11	6	6
	C	6.1	86	10	10	9	3	5	3
	D	6.8	81	15	12 ²	12	7	6	8
	D ¹	8.5	82	23	14 ²	22	12	14	6
	Totals	7.4	—	79	57 ²	58 ³	43	37	33
	In %:				100	91	54	47	42
Basal + Factor 3 ⁴ concen- trate	D	11.1	129	19	0	0	0	0	0
Basal + vitamin E ⁵	E	10.5	114; 188 ⁷	26	0	0	0	0	0
Basal + cystine ⁶	E	12.1	114; 188 ⁸	27	0	25	0	0	1
	In %:				0	93	0	0	4
Basal + vitamin E ⁵ and cystine ⁶	B	14.3	273	19	0	0	0	0	0

¹ Vitamin A and D supplement omitted in these groups.

² There were no survivors. A total of 22 out of 79 animals were sacrificed during the experiment, between the 54th and the 72nd day, for histological and other studies.

³ Incidence of heart lesion not registered in experiment A.

⁴ Factor 3 concentrate prepared from an enzymatic digest of brewer's yeast, equivalent to 40 units of Factor 3 in 100 g of diet³.

⁵ 50 mg% D,L- α -tocopherol acetate.

⁶ 0.5% L-cystine.

⁷ 13 mice killed at 114 days and 13 killed at 188 days after weaning.

⁸ 13 mice killed at 114 days and 14 killed at 188 days after weaning.

certain other selenium compounds, including inorganic selenite¹⁰. In the mouse multiple necrotic degeneration was prevented by 15 μ g of the element (as Na₂SeO₃) in 100 g of the basal diet: All 18 animals of the selenium supplemented group survived for 100 days and were normal at autopsy; a simultaneous control group of 33 mice on the basal ration had an average survival time of 69 days, with no survivors.

The main pathological elements, i.e., heart lesions, liver necrosis, kidney degeneration and muscular dystrophy, which occur *jointly* in multiple necrotic degeneration in the mouse have been observed *separately*,

in different species, on vitamin E deficient diets¹². Heretofore, most of these lesions were attributed solely to lack of tocopherol. Our results show clearly that necrotic degeneration is of multiple dietary origin. It can occur only when both Factor 3 (i.e., biologically active selenium) and tocopherol are missing simultaneously. It is conceivable that the various necrotic lesions result from the disturbance of an essential metabolic mechanism which is dependent on Factor 3 and tocopherol, and common to all organs involved.

¹² K. E. MASON, in W. H. SEBRELL and R. S. HARRIS, *The Vitamins*, Vol. III, 545 (Academic Press, New York, 1954).

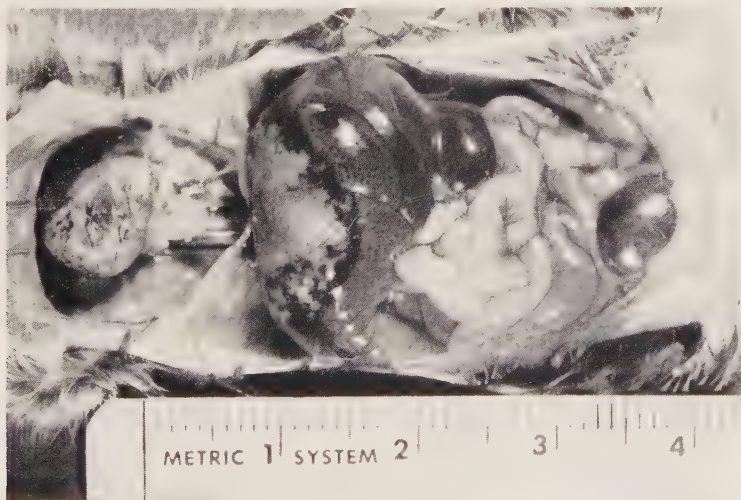


Fig. 1. Multiple dietary necrotic degeneration. Note necrotic lesions of heart and liver, and also discoloration of urine in bladder (hematuria).

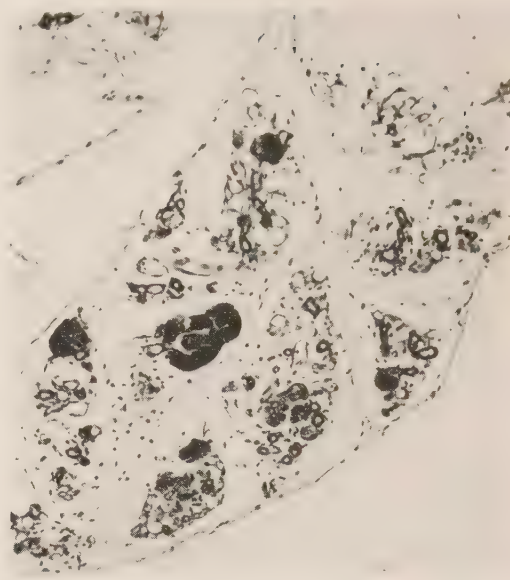
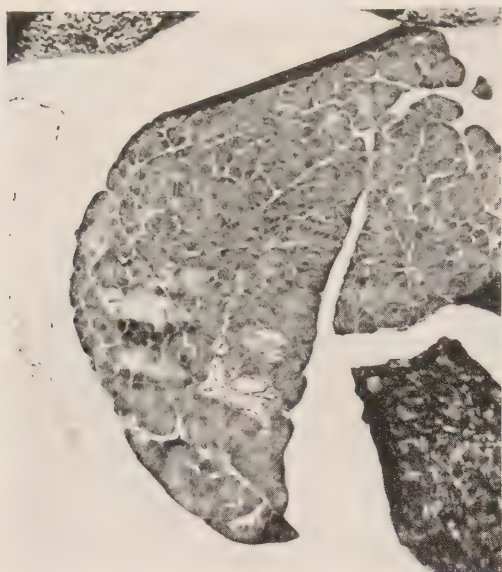


Fig. 2. Pancreatic atrophy. Left: control; right: after 80 days on the basal diet. Paraffin, H. E.

The authors are indebted to Dr. GEORGE L. FITE for histological examinations, and to Mr. RODNEY H. DUVALL for technical assistance.

W. B. DEWITT¹³ and K. SCHWARZ¹⁴

National Institutes of Health, U. S. Public Health Service, Bethesda (Maryland), May 21, 1957.

Zusammenfassung

Bei der Maus wird durch eine Diät eine tödliche Mangelkrankheit, sogenannte multiple, nekrotische Degeneration, erzeugt. Diese Diät ist arm an Cystin und frei von Faktor 3 und Vitamin E. Nekrosen des Herzmuskels stehen im Vordergrund, ausserdem kommt es zu Degeneration von Leber, Nieren und Muskulatur. Pankreas und Testes zeigen Atrophie bei gestörtem Wachstum. Die Krankheit wird entweder durch den Faktor 3 oder durch Vitamin E verhütet. Der Faktor 3, der vor kurzem als eine organische Selenium-Verbindung identifiziert wurde, ist durch Selenit-Selenium ersetzbar.

¹³ Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases.

¹⁴ Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases.

The Effects of Secretin on Urinary Volume and Electrolytes in Normal Subjects and Patients with Chronic Pancreatic Disease

It is well established that intravenous injection of secretin into normal subjects stimulates the release of a large volume of alkaline pancreatic juice containing a high concentration of sodium bicarbonate, whereas in chronic pancreatic disease, this response is altered¹. The Secretin Test, based on these observations, involves

duodenal intubation². In an attempt to avoid the difficulties of intubation, we have investigated changes in urinary excretion of sodium and alkali following secretin injection, in normal subjects and patients with chronic pancreatic disease. Patients were included only if the diagnosis was supported by the most critical clinical and biochemical evidence.

The following experiments were performed:

- (a) 13 Normal Subjects: control injection of normal saline, followed within a week by secretin injection.
- (b) 19 Normal Subjects: secretin injection only.
- (c) 7 Patients with severe chronic pancreatic disease: secretin injection.

Most of the normal subjects were medical students.

Procedure: Day before test: adequate fluid intake (2–3 l).

Day of test. Subject fasting, 7.30 a.m. drinks 100 ml water. Continues to drink this volume at half-hourly intervals throughout the test.

8.30 a.m. Subject at rest in a chair.

9.00 a.m. Subject empties bladder. This specimen is discarded. Test begins: Bladder emptied at 9.20 a.m., 9.40 a.m., 9.50 a.m. and 10.00 a.m. and specimens kept separately under toluene.

10.01 a.m. Injection of test material. Bladder emptied at 10.10 a.m., 10.20 a.m. and 10.30 a.m.

The injection material was either Secretin (Lilly) at a dose of 1 clinical unit/kg body weight, in normal saline, at a concentration of 5 units per ml, or an equal volume of normal saline. Both injections were intravenous, and given over 2 min.

The principal measurements made on the specimens were:

- (1) Volume.
- (2) Sodium – by external standard flame photometry.
- (3) Titratable Acidity and Bicarbonate. Separate estimation of these factors was avoided by using the method of DAWSON *et al.*³, which measures, in a single

¹ G. ÅGREN and H. LAGERLÖF, Acta med. scand. 90, 1 (1936). – D. A. DREILING and H. D. JANOWITZ, Gastroenterology 30, 382 (1956).

² D. A. DREILING, Gastroenterology 24, 540 (1953).

³ J. DAWSON, E. DEMPSEY, F. BARTTER, A. LEAF, and F. ALBRIGHT, Metabolism 2, 225 (1953).

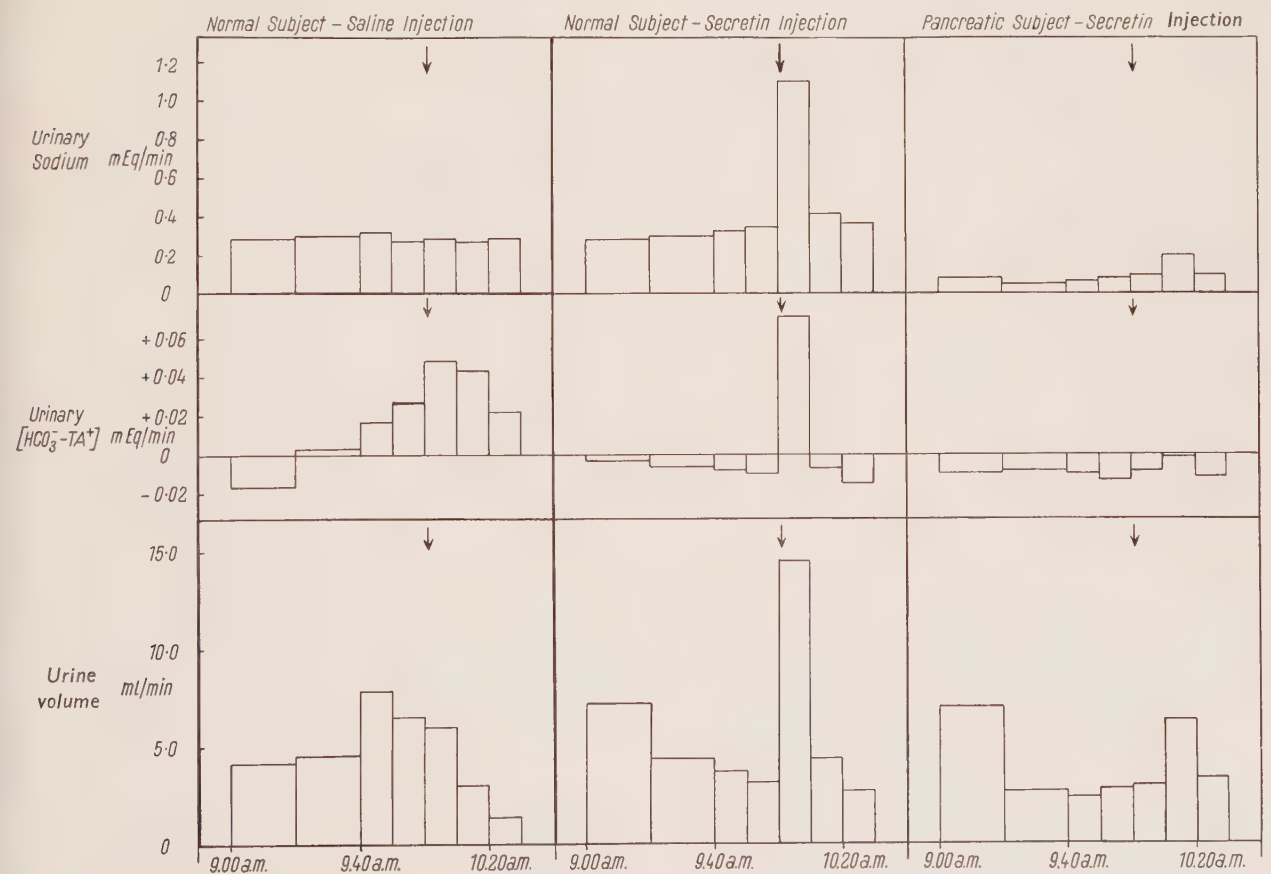
Changes in urinary volume, (HCO_3^- - TA^+) and sodium content in the three groups of experiments. Results are expressed as the *difference* between amounts excreted in the 10 min *before* and the 10 min *after* injection. A 'positive' result indicates that the amount excreted in the 10 min following injection was *greater* than the amount excreted in the 10 min before injection.

Subjects	Urine Excretion - 10 min changes					
	Volume ml		(HCO_3^- - TA^+) mEq.		Sodium mEq.	
	Mean $\pm$ S.D.	Range	Mean $\pm$ S.D.	Range	Mean $\pm$ S.D.	Range
Normal Control saline injection	- 2 $\pm$ 31	- 63 to + 43	- 0.03 $\pm$ 0.19	- 0.45 to + 0.21	+ 0.13 $\pm$ 0.42	- 0.37 to + 0.80
	13 subjects		13 subjects		7 subjects	
Normals Secretin injection	+ 58 $\pm$ 35	- 8 to + 113	+ 0.51 $\pm$ 0.37	0.00 to + 1.42	+ 3.24 $\pm$ 1.98	+ 0.50 to + 6.50
	32 subjects		32 subjects		25 subjects	
Chronic pancreatics Secretin injection	+ 17 $\pm$ 40	- 56 to + 60	- 0.07 $\pm$ 0.20	- 0.33 to + 0.13	+ 1.24 $\pm$ 1.07	- 0.12 to + 2.80
	7 subjects		7 subjects		7 subjects	

operation, titratable acidity minus bicarbonate (TA^+ - HCO_3^-). We have used this measurement, as (HCO_3^- - TA^+), to express urinary acid-base balance. Ammonia should, theoretically, be included in this balance, but we found in early experiments that urinary ammonia levels showed very little variation, despite significant changes in (HCO_3^- - TA^+) and sodium. Using this method, a 'positive' result for (HCO_3^- - TA^+) infers an alkaline

urine, whereas a 'negative' figure infers acidity. Thus, if, before injection (HCO_3^- - TA^+) = - 0.1 mEq., and after injection = + 0.4 mEq., the injection has caused an increase in *alkali* excretion of 0.5 mEq.

(4) pH. No consistant changes were found in early experiments, and this measurement was, therefore, discontinued.



Histograms of typical rates of urinary volume, sodium and (HCO_3^- - TA^+) excretion in (1) normal subjects given (a) saline and (b) secretin; and (2) patients with severe pancreatic disease given secretin. Arrows mark the times at which the injections were given.

Results.—The Figure represents the typical response curves of (1) normal subject given saline, (2) normal subject given secretin and (3) patient with pancreatic disease given secretin. The histograms show that the normal response to secretin is an increase in the urinary volume sodium and alkali excretion, the maximal rates of excretion occurring in the first 10 min following injection. There is a rapid return to normal. The patient with pancreatic disease shows a smaller and delayed volume sodium and alkali excretion, which is maximal in the second or third 10 min period following injection.

The Table summarises the results obtained in the 52 experiments performed. These results are recorded as the *differences* between the quantities excreted in the 10 min period immediately *before* the injection, and the 10 min period immediately *following* the injection.

Normal subjects show a statistically significant difference between control and secretin response with regard to volume, alkali and sodium excretion. The mean increase in the 10 min excretion following secretin is 58 ml urine volume, 0.5 mEq. of alkali and 3.2 mEq. of sodium. Patients with pancreatic disease show significantly less response to secretin with regard to urine volume, alkali and sodium.

As a tentative explanation of these results, we suggest that the initial effect of secretin is the release of pre-formed alkaline pancreatic juice into the duodenum, where both the water and electrolytes are rapidly reabsorbed and then excreted in the urine, giving an 'alkaline tide'.

The validity of this explanation is being further investigated, with special regard to possible actions of secretin on gastric or intestinal secretion or renal tubular function.

The overlap of the ranges of the normal and abnormal response limits the possible application of this test in the diagnosis of pancreatic disease. However, it is likely that if a patient has a maximal normal response severe pancreatic disease can be excluded, whereas a negative response neither proves nor disproves the presence of pancreatic insufficiency. In differentiating between steatorrhea of pancreatic or intestinal origin, this test may, therefore, be helpful, but much further work is required to establish the response in patients with intestinal steatorrhea where the delayed absorption of water and electrolytes may affect the result of the secretin test. In one case of intestinal steatorrhea (non-tropical sprue) investigated so far, a normal response was obtained.

Further work on the physiological basis and possible clinical application of this test is in progress and will be reported at a later date.

Acknowledgements.—We are grateful to the Royal Free Hospital Endowment Fund for generous support and to Eli Lilly and Company for supplying secretin. We would also like to express appreciation to Professor E. M. KILICK and Dr. C. A. B. SMITH for helpful advice, to our clinical colleagues at the Royal Free Hospital and Dr. T. D. KELLOCK of the Central Middlesex Hospital for their cooperation, and to the patients and students for their forbearance.

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and ANN WARRICK*

Department of Chemical Pathology, The Royal Free Hospital, London, October 3, 1957.

* Supported successively by grants from the Royal Free Hospital Endowment Fund.

Zusammenfassung

Injektion von Sekretin ruft bei normalen Versuchspersonen Diuresis und Vermehrung der Natrium- und Alkali-Ausscheidung hervor. Diese Wirkungen sind vermindert bei Patienten mit chronischer Pankreaserkrankung. Der mögliche Mechanismus und die praktische Bedeutung dieser Beobachtungen werden diskutiert.

Primi rilievi sulla presenza di corpi di Heinz durante la crisi emolitica da favismo

Il problema patogenetico della anemia emolitica da favismo è tuttora dibattuto; la maggior parte dei ricercatori dell'ultimo decennio, pur senza aver ottenuto finora dimostrazioni conclusive, si sforza di trovare argomenti in favore di una patogenesi allergica, ed in particolare autoanticorpale.

La dimostrazione da noi raggiunta della presenza di corpi di Heinz nelle emazie durante la crisi emolitica depone in via analogica contro questo modo di vedere. Infatti questi corpi sono stati finora osservati precipuamente in forme emolitiche di natura tossica (anemia da fenilidrazina e derivati, da sulfamidici, da primaquine ecc.).

Noi li abbiamo trovati in 14 su 17 fabici esaminati in fase emoglobinurica, in una percentuale di emazie variante dal 70% al 20% nelle prime 36 h dalla ingestione delle fave, e dal 25% al 0% nella giornata successiva. In 5 casi che al momento del ricovero non presentavano emoglobinuria, o perchè lievi, o perchè già alla fine della crisi, essi erano assenti. In alcuni casi, seguiti nel tempo, si è osservata la progressiva diminuzione e scomparsa dei corpi entro le 48–72 h. In un caso in cui si è potuto seguire il decorso spontaneo della malattia procrastinando la trasfusione di quasi 24 h, si è notato che la diminuzione in valore assoluto delle emazie con corpi di Heinz corrispondeva con sufficiente approssimazione alla quantità totale delle emazie distrutte. Ciò fa pensare che l'azione eritrolesiva delle fave avvenga in un solo tempo e sia quantitativamente determinata fin dall'inizio.

F. PANIZON e G. PUJATTI

Clinica Pediatrica dell'Università di Sassari, il 7 agosto 1957.

Zusammenfassung

Die Verfasser haben Spontaninnenkörper bei 14 von 17 daraufhin untersuchten, an Fabismus leidenden Kindern während des Anfalles gefunden, und zwar innerhalb der ersten 36 h *post ingestionem* der Bohnen in 70–20% der roten Blutkörperchen, am folgenden Tag nur in 25–0%. In einem Fall konnten sie den (durch Bluttransfusion) nicht beeinflussten Verlauf der Krankheit verfolgen und feststellen, dass die Anämie mit dem Schwinden der Innenkörper enthaltenden Blutkörperchen parallel geht. Sie sind daher der Auffassung, dass die blutschädigende Wirkung der Bohnen unmittelbar auftritt und von Anfang an quantitativ festliegt.

Zum Fabismus als Erbkleiden

Der Fabismus ist geographisch streng umschrieben, wird aber trotzdem heute noch eher als eine allergische Erkrankung angesehen.

Vom Material der Universitätskinderklinik Sassari ausgehend, habe ich 100 Familien anamnestisch auf Fabismus untersucht und dabei folgende Tatsachen festgestellt:

1. Das starke Überwiegen des männlichen Geschlechtes, das man am klinischen Material antrifft, ist nur an die grössere Schwere der Erkrankung bei den Knaben gebunden.
2. Die grösste Zahl der Fälle manifestiert sich binnen der ersten fünf Lebensjahre.
3. Bei 77% der Fälle sind beide Eltern normal, bei 21% eines und bei 2% beide gegen die Bohnen überempfindlich.
4. Die gefundenen Frequenzen der befallenen Kinder stimmen gut mit den nach BERNSTEIN berechneten überein, und zwar sind von den Kindern gesunder Eltern $\frac{1}{4}$, von denen eines kranken $\frac{1}{2}$ und von denen beider kranken Eltern alle befallen.

Daraus möchte ich schliessen, dass der Fabismus als ein rezessives Erbleiden anzusehen ist, mit hoher Penetranz und beim männlichen Geschlecht stärkerer Expressivität. Der gefundene Erbgang entspricht nicht dem für die Allergie von anderer Seite vorgeschlagenen.

E. SARTORI

Kinderklinik der Universität Sassari (Italien), 10. August 1957.

Summary

Analysis of 100 families with at last one affected offspring seems conclusive for the assumption that favism is an inherited condition and that the responsible gene is a recessive one. The penetrance of the gene seems to be high and the expression stronger in males.

Antimalarials in Rheumatoid Arthritis

During the last few years a number of authors have described the use of antimalarials in the treatment of rheumatic disorders¹.

¹ Lancet 1957/I, 414. – J. ESCARPENTIER-ORIOI, A. CARRERAS-BAYÉS, and M. SARIOLS-GOMEZ, Medizinische 1955, 1083. – J. FORESTIER and A. CERTONCINY, Rev. Rhum. 21, 395 (1954). – A. FREEDMAN, Ann. Rheum. Dis. 15, 251 (1956). – A. FREEDMAN and F. BACH, Lancet 1952/II, 321. – CH. GRUPPER, Bull. Soc. franç. Derm. 5, 423 (1954). – G. G. HAYDU, Amer. J. med. Sci. 225, 71 (1953). – W. HULL and L. PARR, VIIte Congrès International du Rhumatisme, Genève 1953. – J. LACAPÈRE, H. MONIER, and G. VIAL, Rev. Rhum. 21, 389 (1954). – X. VILANOVA, Klin. Wschr. 32, 905 (1954).

Recent pharmacological investigations on animals throw some light upon these clinical results. We described previously² experiments in which mepacrine, proguanil and primaquine were examined in regard to their action on the formaline-arthritis of rats; the results obtained indicated that these antimalarials possess an intensive unspecific antiinflammatory action. We believe that this quality is in question when rheumatic disorders, lupus erythematosus, etc., are treated with antimalarials. Our earlier animal experiments were carried out under anesthesia (100 mg/kg subcutaneously of Numal ‘Roche’). We have now investigated the effects of mepacrine and chloroquine on unanesthetized animals. For each substance 10 or 20 animals were used. Mepacrine or chloroquine were given by mouth, and, exactly 30 min later, inflammation was induced by the subcutaneous injection of 0.1 ml of 3% formalin into a hind paw. Exactly 135 min after the injection, the animals were killed; the paw and its uninflamed fellow were immediately amputated and their volumes determined. The difference of these volumes gave the volume of swelling caused by the irritant. The anti-inflammatory efficacy of the antimalarials tested was estimated by comparison of the volume of swelling in these animals with that similarly produced in control animals which had not received any anti-inflammatory treatment.

The results are summarized in the Table. They indicate, that the unspecific inhibition of inflammation by antimalarials as well in unanesthetized as in anesthetized animals corresponds to the anti-inflammatory intensity of cortisone under the same conditions.

R. DOMENJOZ and W. THEOBALD

Pharmacological Laboratories, J. R. Geigy, Basle, September 16, 1957.

Zusammenfassung

Mit Malariamitteln konnte am Formalinödem der Ratte in einer Dosis von 50 mg/kg *per os* sowohl bei narkotisierten als auch bei nichtnarkotisierten Tieren eine unspezifische entzündungshemmende Komponente nachgewiesen werden. Die Intensität der inhibitorischen Wirkung entsprach dabei der Entzündungshemmung von 2×50 mg/kg Cortison. Unserer Ansicht nach dürfte dieser unspezifische antiphlogistische Effekt bei der Behandlung rheumatischer Affektionen, des Lupus erythematosus usw. mit Malariamitteln zur Geltung kommen.

² R. DOMENJOZ, Sem. Hôp. Paris 31, 2703 (1955). – W. THEOBALD and R. DOMENJOZ, Dtsch. med. J. 6, 53 (1955).

‘Formaline oedema’ of rats

Substances	Under anesthesia				Without anesthesia		
	Cortisone	Mepacrine	Chlorguanid	Primaquine	Cortisone	Mepacrine	Chloroquine
Doses in mg/kg orally		50	50	50		50	50
Subcutaneously	2×50				2×50		
Number of animals	20	20	20	20	20	10	20
Volume of swelling in mm ³ . .	124	115	116	123	191	216	211
$\sigma \pm$	78.28	48.69	47.88	55.40	81.68	68.95	61.94
$\varepsilon \pm$	17.50	10.89	10.71	12.38	18.26	21.81	13.85
Significant Difference.	6.32	8.44	8.44	7.60	3.85	6.1	4.63
Inhibition in % in comparison to untreated controls . .	54	57	57	54	33	38	37

PRO EXPERIMENTIS

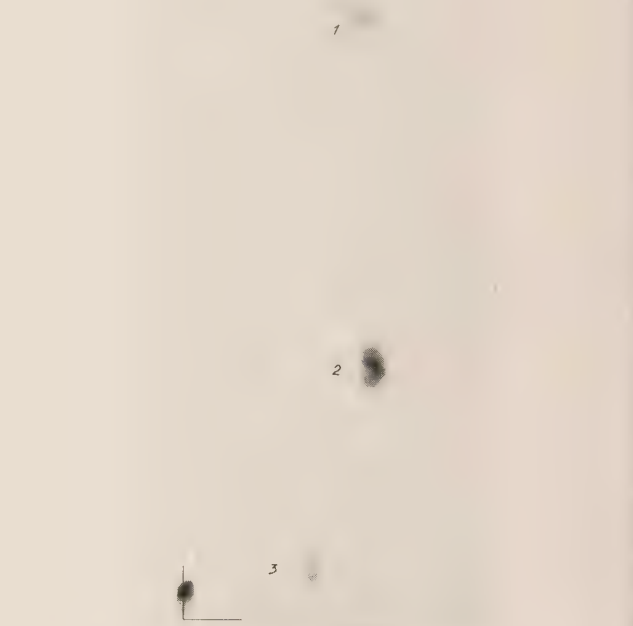
Identificazione cromatografica della fosforil-colina, -etanolamina, -serina, della α -gliceril-fosforil-colina, -etanolamina e della citidindifosfatocolina nell'encefalo di ratti in accrescimento

Dal sistema nervoso centrale sono stati estratti alcuni corpi fosforati liberi, partecipanti verosimilmente al metabolismo dei fosfatidi in qualità di precursori dei medesimi od a vicenda di prodotti di loro degradazione. Essi sono: la fosforiletanolamina¹ e la glicerilfosforiletanolamina²; è stata anche segnalata la presenza della fosforilcolina³ e della glicerilfosforilcolina⁴. In questi ultimi tempi inoltre sono state individuate nel fegato di ratto piccole quantità di citidindifosfatocolina e citidindifosfoetanolamina⁵; secondo gli autori questi citidincomposti partecipano alla sintesi dei fosfatidi, contenenti colina ed etanolamina, quali immediati precursori. Infine è stato possibile separare dal sistema nervoso centrale mediante cromatografia su carta singoli fosfatidi alcali-labili (fosfatidilcolina, fosfatidiletanolamina e fosfatidilserina⁶) e studiare *in vivo* in quel apparato la loro velocità di «turnover» durante i primi giorni di vita del ratto e nelle sue età corrispondenti a 100 g di peso⁷.

Lo scopo della presente ricerca è stato quello di separare da uno stesso piccolo campione di tessuto nervoso il maggiore numero di composti citati sopra e di studiare *in vivo* la velocità di «turnover» di ciascuno di essi. Con ciò fu possibile precisare la posizione da loro occupata nel ciclo metabolico di alcuni fosfatidi.

Un encefalo di ratto di 3 oppure di 13 giorni di vita (340–610 mg) veniva frammentato per 2 min in un piccolo omogenizzatore (4000 $\times$ g), contenente 6 ml di H₂O bollente. La frammentazione era continuata per altri 5 min sostituendo il bagno-maria bollente con acqua ghiacciata. L'omogenato e l'acqua di lavaggio dell'omogenizzatore erano raccolte in una provetta e centrifugate per 15 min a 4000 $\times$ g. Il sopranatante di colore citrino e leggermente opaco veniva filtrato attraverso carta da filtro S.S. 589/2 $\varnothing$ 7 cm e raccolto in una provetta, contenente 600 mg di Norit A precedentemente lavata con N HCl. Alla fine della filtrazione la carta da filtro e l'esiguo precipitato della centrifugazione venivano accuratamente lavati con H₂O, la quale era poi aggiunta al filtrato. 10 ml di metanolo-cloroformio, 1/2, erano versati nella provetta contenente il precipitato, previa aggiunta della carta da filtro (su di essa infatti si è stratificata durante la filtrazione una pellicola di frammenti contenenti lipidi). In tale modo fu possibile estrarre una quantità di fosfatidi alcali-labili sempre sufficiente per la loro successiva individualizzazione (frazione A).

Il filtrato veniva di tanto in tanto agitato affinché la Norit restasse in sospensione e l'operazione era continuata per 7–8 ore. Durante questo periodo il filtrato diventa limpido; alla fine esso veniva centrifugato ed il sopranatante raccolto in un palloncino. La Norit veniva lavata a sua volta con H₂O e centrifugata. Il sopranatante di quest'ultima era riunito al primo e l'insieme veniva liofilizzato (frazione B).



Autoradiogramma di composti contenenti ³²P (frazione B), estratti da un encefalo di ratto di 88 ore di vita e separati mediante cromatografia su carta; l'animale è stato sacrificato dopo 16 ore dalla somministrazione di NaH₂³²PO₄. Composti: 1) fosforilcolina; 2) fosforiletanolamina; 3) fosforilserina. Tempo di esposizione: 21 giorni.

10 ml di piridina al 10% venivano aggiunte alla Norit e la provetta era agitata di tanto in tanto per 20 min. Dopo centrifugazione il sopranatante veniva raccolto in un separatore, previa filtrazione attraverso carta S.S. 589/1 $\varnothing$ 7 cm. La Norit era nuovamente trattata con 5 ml di piridina al 5% per 10 min, poi centrifugata ed il sopranatante raccolto per filtrazione nello stesso separatore. Per eliminare la piridina il filtrato veniva sbattuto energicamente con 25 ml di cloroformio e ciò per tre volte, rimuovendo ogni volta la fase contenente cloroformio. Alla fine la fase acquosa e l'acqua di lavaggio del separatore erano centrifugate ed il sopranatante limpido era raccolto in un palloncino assieme all'acqua di lavaggio del precipitato; il tutto alla fine era liofilizzato (frazione C).

Dalla frazione A, previa blanda idrolisi alcalina dei fosfatidi⁸, erano separati mediante cromatografia su carta i frammenti fosfodiesterici della fosfatidilcolina, fosfatidiletanolamina e fosfatidilserina⁷.

La frazione B era ridisciolta in 0,3–0,4 ml di H₂O; da essa erano prelevati 0,01 ml mediante microsiringa micrometrica Agla e depositati su carta per cromatografia Whatman n° 1 precedentemente lavata con 2N ac. acetico. Per la prima corsa (18 h) è stato usato il solvente

¹ W. E. STONE, J. biol. Chem. 149, 29 (1943). – J. AWAPARA, A. J. LANDUA e R. FUERST, J. biol. Chem. 183, 545 (1950). – G. B. ANSELL e R. M. C. DAWSON, Biochem. J. 50, 241 (1951).

² G. B. ANSELL e J. M. NORMAN, Biochem. J. 55, 768 (1953).

³ R. M. C. DAWSON, Biochem. J. 60, 325 (1955).

⁴ R. M. C. DAWSON, Biochemistry of the developing nervous system (Acad. Press, New York 1955), p. 268.

⁵ E. P. KENNEDY e S. B. WEISS, J. biol. Chem. 222, 193 (1956).

⁶ R. M. C. DAWSON, Biochim. biophys. Acta 14, 374 (1954). – N. MIANI, Boll. Soc. ital. Biol. sper. 31, 1008 (1955).

⁷ N. MIANI, Biochim. biophys. Acta (in stampa).

⁸ R. M. C. DAWSON, Biochim. biophys. Acta 14, 374 (1954).

fatto di fenolo/acqua, 80/20, (*p/v*), e per la seconda corsa (10 h) etanolo/ $\text{NH}_3/\text{H}_2\text{O}$, 60/20/10, o più raramente collidina/lutidina/acqua, 100/100/100. Sulla carta sono state individuate: la fosforilcolina, la fosforiletanolamina, la fosforilserina, la $\text{L-}\alpha$ -glicerilfosforilcolina, e la $\text{L-}\alpha$ -glicerilfosforiletanolamina.

La frazione C era ridisciolta in 0,2–0,3 ml di H_2O ; da essa erano prelevati 0,005–0,008 ml e depositati su carta per cromatografia S. S. 2045/a precedentemente lavata con 2*N* ac. acetico. Per la prima corsa (10 h) è stato usato il solvente fatto di acetone/0,005 *N* ac. acetico, 50/50, e per la seconda corsa (20 h) il solvente di KREBS e HEMS¹⁰ senza versene (ac. isobutirrico/*N* NH_3 , 100/60). Sulla carta è stata finora identificata, fra gli altri nucleotidi, la citidindifosfatocolina.

I dati ottenuti possono essere così riassunti:

1° È stato possibile separare contemporaneamente da piccole quantità di tessuto nervoso (340–610 mg) un notevole numero di corpi fosforati liberi (alcuni già conosciuti, altri poco o non conosciuti), i quali partecipano verosimilmente al metabolismo di alcuni fosfatidi in qualità di precursori dei medesimi od a vicenda di prodotti di loro degradazione.

2° Essi sono: la fosforiletanolamina e la $\text{L-}\alpha$ -glicerilfosforiletanolamina (già conosciuti)¹¹, la fosforilcolina e la $\text{L-}\alpha$ -glicerilfosforilcolina (poco conosciuti)¹², la fosforilserina e la citidindifosfatocolina (non conosciuti). Inoltre sono state separate dallo stesso campione di tessuto la fosfatidil-colina, -etanolamina e -serina.

3° Tutto lascia supporre che altri corpi fosforati, specificatamente la glicerilfosforilserina, la citidindifosfato-etanolamina e -serina ancora non identificati siano presenti sui cromatogrammi.

4° Sulla base di prove collaterali sembra che il metodo di estrazione e di frazionamento dell'estratto acquoso (frazioni B e C) non alteri, nei limiti dell'errore sperimentale, qualitativamente e quantitativamente i composti fosforati ricercati.

5° La concentrazione media (76 animali) di alcuni composti fosforati nell'encefalo di ratti compresi fra il 3° ed il 13° giorno di vita è così risultata: fosforilcolina $76 \pm 13 \mu\text{g P/g}$ di tessuto fresco; fosforiletanolamina $91 \pm 7 \mu\text{g P/g}$; fosforilserina $29 \pm 8 \mu\text{g P/g}$; $\text{L-}\alpha$ -glicerilfosforilcolina $42 \pm 14 \mu\text{g P/g}$; $\text{L-}\alpha$ -glicerilfosforiletanolamina $32,4 \pm 11 \mu\text{g P/g}$. Nel calcolo non è stato tenuto conto delle perdite che ciascun composto subisce durante la separazione cromatografica su carta.

N. MIANI

Istituto di Anatomia, Università di Padova, il 16 Settembre 1957.

Summary

A method is described whereby the phosphorylcholine, -ethanolamine, -serine, the $\text{L-}\alpha$ -glycerylphosphorylcholine, -ethanolamine, the phosphatidylcholine, -ethanolamine, -serine and also the cytidinediphosphatecholine may be separated in the brain of a rat a few days old (340–610 mg).

⁹ R. M. C. DAWSON, *Biochem. J.* **62**, 693 (1956).

¹⁰ H. A. KREBS e R. HEMS, *Biochim. biophys. Acta* **12**, 172 (1953).

¹¹ W. E. STONE, *J. biol. Chem.* **149**, 29 (1943). – J. AWAPARA, A. J. LANDUA e R. FUERST, *J. biol. Chem.* **183**, 545 (1950). – G. B. ANSELL e J. M. NORMAN, *Biochem. J.* **55**, 768 (1953).

¹² R. M. C. DAWSON, *Biochem. J.* **60**, 325 (1955); *Biochemistry of the developing nervous system* (Acad. Press, New York 1955), p. 268.

PRO EXPERIMENTIS

Electrophoretic Analysis of Clinical Dextrans

The clinical value of dextran depends on its molecular weight. The mean molecular weight, however, is a very unreliable index of the therapeutic properties unless supplemented by the analysis of the polydispersity of the dextran preparation.

We have recently shown that dextran dissolved in a borate buffer exhibits electrophoretic mobility and that a mixture of two homogeneous dextran fractions of different molecular weight can be partitioned in the electric field with a good recovery of each component¹.

It appears that electrophoresis of dextran may afford means of estimating both the mean molecular weight and the polydispersity of a dextran preparation.

Materials and Methods.—*Electrophoresis* was carried out in a Fokal-B apparatus (Strübin & Co., Basle) at + 2.5° C, using white light and a standard 85 mm Tiselius type cuvette². The percentage composition of a mixture was evaluated by fitting Gaussian curves to the enlarged patterns.

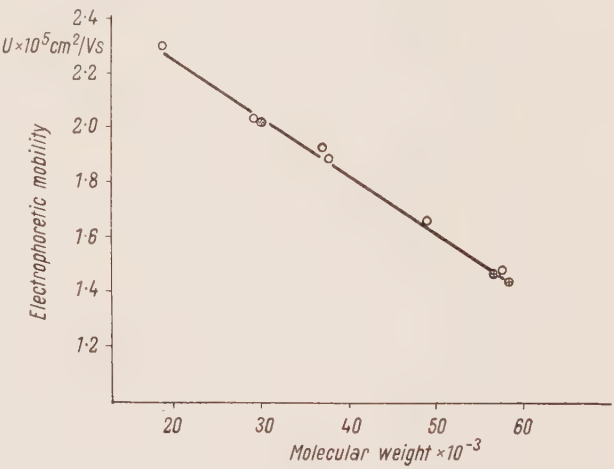


Fig. 1.

Borate buffer was prepared by adjusting a boric acid solution to pH 10.0 (glass electrode) with sodium hydroxide; the final concentration of borate ion was 0.05 *M* in all experiments.

Molecular weights were calculated from the combined diffusion constant, intrinsic viscosity and partial specific volume determinations³.

Purified dextran fractions were obtained from acid-hydrolysed native dextran by repeated ethanol fractionation. The final lyophilised preparations were dissolved in the borate buffer and exhaustively dialysed against the same buffer.

Results.—Electrophoretic mobilities of purified dextran fractions were determined; the mean values, calculated from ascending and descending boundaries, are plotted against molecular weights of the fractions as shown in Figure 1. The data on 'Macrodex'-fractions, kindly donated by Pharmacia A.G., Uppsala, Sweden, are included in this graph and marked with crosses. It is evident that electrophoretic mobility is linearly de-

¹ K. ZAKRZEWSKI, Z. MAY, and K. MURAWSKI, *Biokhimija USSR*, **21**, 596 (1956).

² E. WIEDEMANN, *Exper.* **3**, 341 (1947).

³ K. ZAKRZEWSKI, J. KRYSIAK, K. MURAWSKI, Z. MAY, and J. MALEC, *Bull. Acad. Polonaise Sci. Cl. II* **2**, 67 (1954).

Preparation	Intrinsic viscosity + 25°, 0.16 M NaCl	Electrophoresis results						Pattern in Fi- gure 2
		Component I			Component II			
		mobility $U \cdot 10^5$ $\text{cm}^2/\text{V} \cdot \text{s}$	molecular weight $\times 10^{-3}$	%	mobility $U \cdot 10^5$ $\text{cm}^2/\text{V} \cdot \text{s}$	molecular weight $\times 10^{-3}$	%	
,Intradex' Benger Labs. England	0.22	1.14	> 60.0	21.8	1.76	46.6	78.2	<i>a</i>
,Dextraven' Benger Labs. England; Nr. 18055 .	0.26	0.99	≥ 60.0	65.0	1.56	53.8	35.0	<i>b</i>
,Macrodex' Pharmacia AG., Sweden; A7673 A .	0.24	1.41	61.0	79.2	2.36	16.0	20.8	<i>c</i>
,Expandex' Com. Solv. Corp., U.S.A. Nr. 36610 A	0.20	1.59	52.4	100.0	—	—	—	<i>d</i>
,Gentran' Baxter Labs. U.S.A., Nr. 94090 . . .	0.22	1.11	> 60.0	100.0	—	—	—	<i>e</i>
,Poliglukan' Experimental preparation, Poland .	0.19	1.49	57.0	100.0	—	—	—	<i>f</i>

pendent on molecular weight, at least within the molecular weight range of about 18,000 to about 60,000. This relationship has been made use of in determination of molecular weights of investigated dextrans described below.

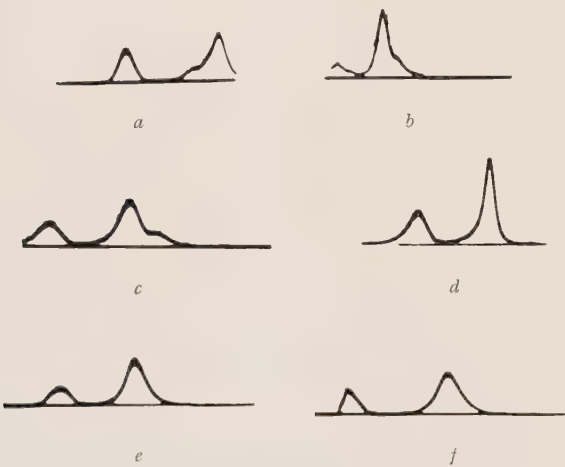


Fig. 2.

Samples of clinical dextrans of various manufacturers obtained from commercial sources, were analysed under the same conditions. Typical electrophoretic patterns are presented in Figure 2 *a-f*, and the results of corresponding calculations in the Table.

The above results clearly point to the usefulness of electrophoresis in the analysis of clinical dextrans.

Z. MAY and K. ZAKRZEWSKI

Department of Biochemistry, Institute of Haematology, Warsaw (Poland), July 26, 1957.

Résumé

On démontre que l'électrophorèse de dextrans cliniques peut être employée pour la détermination de leur poids moléculaire et de la polydispersité.

PRO LABORATORIO

Techniques for Making Capillary Microelectrodes¹

The use of the LING-GERARD capillary microelectrodes² in electrobiological research has been rapidly growing. While microelectrodes can be made by hand and commercially made capillary pullers are available, a puller which can be assembled from parts found in a laboratory would have wide usefulness. A technique for rapidly filling capillaries is also described.

The principle of the puller is simple. A short length of soft glass tubing (about 1 mm o.d.) is held in the jaws of a tong-like holder. The tongs are so constructed that approximation of the handles causes the jaws to separate. An elastic band is stretched across the handles, but the jaws are prevented from separating by the capillary tubing. The tubing is heated with a microflame and the softened glass pulled to a fine capillary tip as the result of the restoring force of the elastic band. Simultaneously, with the sudden release of tension, the microflame is automatically pushed aside.

A detailed description of the component parts follows: A tong-like holder (*A*) may be adapted from any tongs whose arms do not cross over at the pivot point, or may be fashioned from small stock. The ends of the tongs with the wider excursion (of about 4 inches) are fitted with alligator clamps (*B*) in the jaws of which are placed thin strips of rubber. One arm of the tongs is fixed by a clamp, while the other arm is free to move. With the capillary tubing (*C*) inserted, the alligator clamps are approximately 1–2 inches apart. A rubber band (*D*) is looped several times across the opposite ends of the tongs so as to be under stretch when the capillary tubing is in place.

The microburner (*E*) is made from a 13 gauge hypodermic needle flared at the point to simulate a wing tip. The wing tip is made by cutting off the bevel of the needle and pinching the ends with a pair of pliers. The microburner is clamped to a horizontally oriented rod

¹ This work was supported by a research grant from the National Institute of Neurological Diseases and Blindness, U. S. Public Health Service.

² G. LING and R. W. GERARD, *J. cell. comp. Physiol.* **34**, 382 (1949).

bent at 120 degrees and the other end of the rod fixed to a pulley (*F*) to provide movement in the horizontal plane. The wing tip is oriented along the long axis of the capillary.

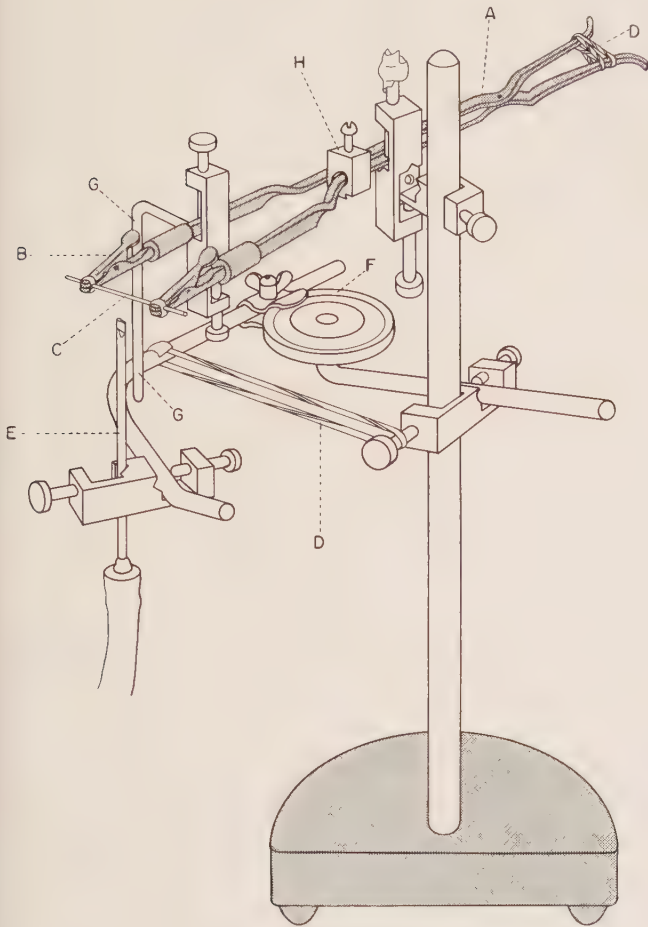


Diagram of micropipette puller.
A Tong like holder, *B* Alligator clamp, *C* Capillary tubing, *D* Rubber band, *E* Microburner, *F* Pulley, *G* Vertical rod, *H* Movable stop

A vertical rod (*G*) is fixed to the movable arm of the tongs through a 90° angle and rides in the angle of the horizontally oriented rod holding the microburner. The vertical rod functions as a stop for positioning the microburner and also pushes the microburner aside in the final stage of pulling. In order to align the flame consistently with respect to the capillary tubing, a rubber band (*D*) may be attached between the horizontal rod and a clamp on the stand. A movable stop (*H*) on the stationary arm of the tongs is a convenient aid in the initial alignment of flame and capillary tubing.

The size of the flame and its distance from the tubing seem to be the only critical variables. In general, a small blue cone flame gives the most satisfactory results. Electrodes of less than 0.5 μ can be fabricated routinely.

The filling of capillary micropipettes under reduced pressure has been in use for some time³. It appears worthwhile to describe in detail a simple filling technique which does not seem to be widely known. The capillaries in a plastic rack are held vertically with tips down and shank ends covered by filtered 3 *M* KCl. The top of the

rack is a flat disc of the same diameter as the containing vessel, a heavy-walled Pyrex cylindrical jar (4" $\times$ 6"), filled to a level about $\frac{2}{3}$ capacity. The cuff of a large sized heavy rubber glove is fitted on the wide end of a Büchner funnel and then stretched down over the Pyrex jar to give a vacuum seal. The solution is heated to almost boiling and reduced pressure then applied from a water pump while the heating is continued. After 5 min air is admitted to the system. It is important that the electrodes are completely immersed in solution at all times during the filling process. Cooling to about 4°C will usually eliminate a small air space which may remain occasionally in an electrode.

S. E. KIRK and G. FALK

Department of Pharmacology, University of Washington, School of Medicine, Seattle (Washington), July 11, 1957.

Résumé

Nous décrivons un appareil facile à construire pour étirer des microélectrodes capillaires et une manière simple de remplir ceux-ci sous pression réduite.

PRO LABORATORIO

A Perspex* Moist Chamber for Micromanipulation

A modification of the moist chamber described by FOWELL¹, for the isolation of yeast ascospores and vegetative cells has been constructed from perspex. Incorporated in the design is an easily removable lower floor of glass. This facilitates initial inoculation, final isolation and any intermediate macromanipulation that may be required, without the removal of the chamber roof, on the underside of which the micromanipulation has been carried out.

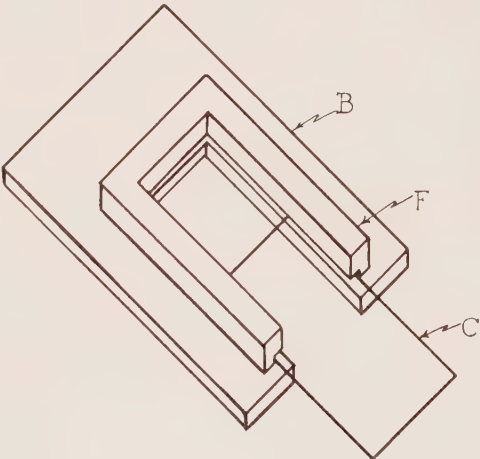


Fig. 1.

The U shaped frame (*F*) is made from $\frac{1}{4}$ in. square section perspex and is mounted on the base (*B*) made from $\frac{1}{8}$ in. sheet material. The portion of the base lying

³ R. D. KEYNES and H. MARTINS-FERREIRA, *J. Physiol.* 119, 315 (1953).

* = Plexiglas.
¹ R. R. FOWELL, *J. appl. Bact.* 18, 149 (1955).

between the arms of the frame is cut away. The lower inner edge of the frame is rebated to enable a $2 \times \frac{15}{16}$

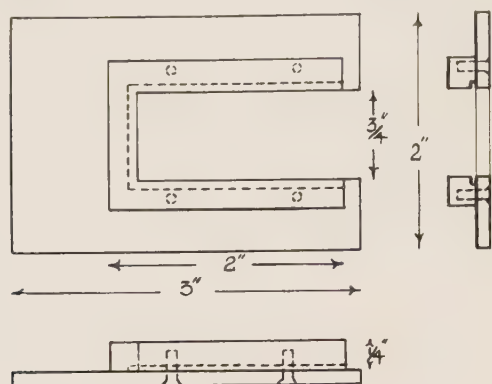


Fig. 2.

in. coverslip (C) to be inserted and act as the floor of the chamber. The roof consists of two $\frac{7}{8}$ in. coverslips or a single $2 \times \frac{15}{16}$ in. coverslip these being lightly cemented in position with small spots of a lanolin-resin cement. Inoculation, final isolation, and other macro-manipulation is carried out by sliding out the basal coverslip to the required distance.

P. A. DIXON

Birkbeck College, University of London, March 19, 1957.

Résumé

Une chambre humide pour la micromanipulation a été construite en Perspex. Elle est munie d'un plancher inférieur mobile en verre, qui facilite l'inoculation initiale, la macromanipulation intermédiaire et l'isolement final.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

The Metabolic Products of *Penicillium patulum* and their Probable Interrelationship¹

By E. W. BASSETT and S. W. TANENBAUM²

During the course of a study of the biosynthesis of patulin in *Penicillium urticae*, Bainier (syn. *P. patulum*) strain 2159A³, several closely related compounds were found to accumulate in the growth filtrate. Many of the metabolic products had previously been identified, including gentisic acid, gentisaldehyde, and gentisyl alcohol by BIRKENSHAW *et al.*⁴, and 6-methylsalicylic acid by EHRENSVÄRD⁵. In our experiments using this nonpigmented mutant strain, the following additional compounds have been identified: 6-formylsalicylic acid, 3-hydroxyphthalic acid, pyrogallol, *p*-hydroxybenzoic acid, anthranilic acid, and an aliphatic substance which we have tentatively named 'pre-patulin'.

Pathways for the biosynthesis of patulin have been proposed first by BIRKENSHAW⁶ and later by EHRENSVÄRD⁷. Their salient features involve the oxidative rupture of gentisaldehyde followed by rearrangement and closure to patulin. EHRENSVÄRD's scheme places

6-methylsalicylic acid as the product of a side reaction and does not recognize a direct relationship between this C₈ metabolite and the C₇ aromatics postulated as patulin precursors. With the finding of 6-formylsalicylic acid and 3-hydroxyphthalic acid, heretofore unrecorded in biological systems, we are able to fit all of the known metabolic products of this mold into a logical sequence.

Results.—The identification of 6-formylsalicylic acid (6-FSA) was made by comparison with authentic⁸ material synthesized by the method of ELIEL, RIVARD, and BURGSTÄHLER⁹. A list of the physical and chemical characteristics of the isolated and authentic materials is given in the Table (p. 40). The ultraviolet absorption spectra are presented in the Figure. By comparison with synthetic material prepared from 3-aminophthalic acid, 3-hydroxyphthalic acid was identified. Both the synthesized and isolated compounds had the same properties, i.e., m.p. 154°, $\lambda_{\max} = 323 \text{ m}\mu$, as well as identical paper chromatographic and color reactions. Further fractionation of the culture filtrates afforded pyrogallol in good yield (m.p. 134°, m.p. of triacetate 162°); while *p*-hydroxybenzoic acid and anthranilic acid were detected by paper chromatographic and spectrophotometric analysis. These last two acids have long been known to be intimately related to aromatic biosynthesis from shikimate both in *Aerobacter*¹⁰ and in *Neurospora*¹¹. To the authors' knowledge, pyrogallol, the classic example of the higher plant aglycones, has not previously been found among the fungi.

From cultures grown on glucose in the presence of CaCO₃ and which failed to show evidence of patulin formation by the usual criteria, a waxy material ($\lambda_{\max}$

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³ We should like to thank Dr. C. W. HESSELTINE of the NRRL, Peoria, Ill., for sending us this culture.

⁴ J. H. BIRKENSHAW, A. BRACKEN, S. A. MICHAEL, and H. RAISTRICK, *Lancet* 245, 625 (1943). — J. H. BIRKENSHAW, A. BRACKEN, and H. RAISTRICK, *Biochem. J.* 37, 726 (1943).

⁵ G. EHRENSVÄRD, *Exp. Cell Res.*, Suppl. 3, 102 (1955).

⁶ H. J. BIRKENSHAW, *Ann. Rev. Biochem.* 22, 371 (1953).

⁷ J. H. BIRKENSHAW, A. BRACKEN, and H. RAISTRICK, *Biochem. J.* 37, 726 (1943).

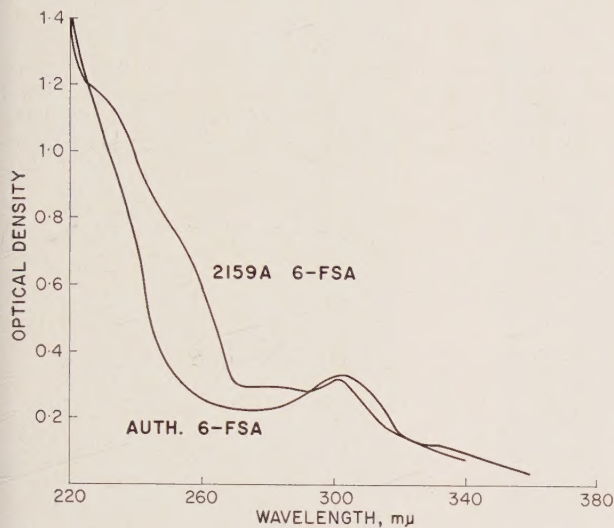
⁸ We are indebted to Prof. E. L. ELIEL for a generous supply of this compound.

⁹ E. L. ELIEL, D. E. RIVARD, and A. W. BURGSTÄHLER, *J. org. Chem.* 18, 1679 (1953).

¹⁰ B. D. DAVIS, *J. Bact.* 64, 729 (1952).

¹¹ E. L. TATUM, S. R. GROSS, G. EHRENSVÄRD, and L. GARN-JOBST, *Proc. nat. Acad. Sci., Wash.* 40, 271 (1954).

($\approx 280 \text{ m}\mu$) was obtained. This substance was free of aromatic phenolic acids and reacted readily with 2,4-dinitrophenylhydrazine to give a derivative with an R_f and melting point which differed from those for patulin-2,4-dinitrophenylhydrazone. Yet, this waxy substance could be converted to patulin by recrystallization in anhydrous solvents with a trace of acid. Conversely, patulin, by manipulation with mild base was transformed into the uncharacterized material. We have reason to believe therefore that this product of blocked metabolism is the open chain precursor of the antibiotic.



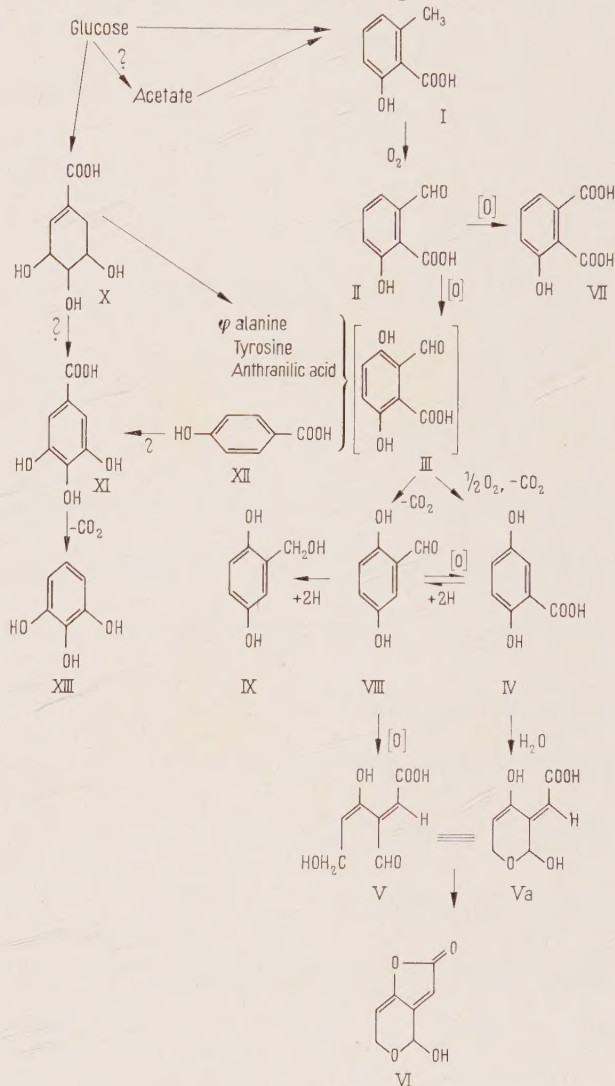
Finally, we wish to record at this time an interesting neutral substance (m.p. 218–219°, $\lambda_{\max} = 292 \text{ m}\mu$) which has been isolated from *P. patulum* 2159A broth. This substance migrates as a single spot in several chromatographic systems, and upon acid hydrolysis gives rise to 6-methylsalicylic and 6-formylsalicylic acids. The molecular weight by the Rast method gives a value of 326, which is not inconsistent with values calculated for various combinations of these two moieties in depsidic, depsidone, or diester linkages. The exact chemical nature of this material is under investigation.

Following growth of strain 2159A on Czapek-Dox medium with 4% glucose at room temperature, maximum patulin accumulation occurred on the seventh to ninth day. The following acidic carbonyl compounds could be detected in the filtrate and were separated and identified as their 2,4-dinitrophenylhydrazones: pyruvic, glyoxylic, α -ketoglutaric, and oxaloacetic acids, and 'pre-patulin'. The first component to appear on the second day of growth in cultures with CaCO_3 and glucose was 6-methylsalicylic acid; this substance increased steadily in concentration until the sixth day, at which time gentisic acid began to accumulate and 6-methylsalicylate diminished progressively. At this point gentisaldehyde and 'pre-patulin' were also present in high concentration, probably owing to the trapping of these C₇ acids as their calcium salts.

In replacement experiments with eight-day-old exhaustively washed mycelial pads, glucose readily gave rise to patulin within 48 h. Glucose-grown pads converted acetate exclusively to 6-methylsalicylic acid in this interval. In turn, replacements with 6-methylsalicylate as substrate formed gentisic acid, gentisaldehyde, 3-hydroxyphthalic acid, and patulin. The replacement experiments with gentisaldehyde produced patulin and gentisic acid in small yield, with most of the substrate

decomposing to dark colored oxidation products. However, gentisic acid was converted promptly to patulin by this technique. With shikimic acid as supplement, *p*-hydroxybenzoic acid was the sole detectable aromatic substance.

The first indication of the effect of trace metal concentration on the proportion of metabolic products from *P. patulum* came from the experiments of BRACK¹². Similar experiments in this laboratory with strain 2159A have shown that cobalt and manganese at 10^{-6} M favor patulin formation, whereas zinc in this concentration causes the equilibrium to shift towards 6-methylsalicylic acid accumulation. Again, when the iron concentration in Czapek-Dox medium is reduced to 1 mg $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O/l}$, reduction in yield of patulin occurs with concurrent increase of the C_7 and C_8 aromatic acid concentration. Contrary to the results of BRACK¹² and of EHRENSVÄRD⁵, neither gentisyl alcohol nor toluquinol was detected in any of the above experiments.



Discussion.—By consideration of the chemical nature of the substances found in the growth filtrate and from the sequence in which they have appeared in growing and in replacement cultures, the following scheme is proposed for their biogenetic interrelationship (Formula).

¹² A. BRACK, *Helv. chim. Acta* 30, 1 (1947).

Comparison of 6-FSA Properties

	Isolated	Authentic
m.p.	134°	134–137° (134° mixed)
FeCl ₃ Test	red	red
*R _f (Uvl. Color)	(a) 0.60 (yel) (b) 0.82 (bl)	(a) 0.60 (yel) (b) 0.82 (bl)
Diazo Spray	Light brown	Light brown
2,4-dnp m.p.	265°	272° (265° mixed)
*R _f 2,4-dnp	0.75	0.75

* BuOH–0.5 N NH₄OH–EtOH 70/20/10

Thus, we envision that aromatization takes place first either from glucose or from activated acetate to give 6-methylsalicylic acid (I), which in turn by a two step oxidation at the methyl group becomes 6-formylsalicylic acid (II). The finding of 3-hydroxyphthalic acid (VII) in filtrates and in replacement media is taken simply as a result of further oxidation of (II) at the formyl group, which yields an additional carboxyl. The hypothetical 5-hydroxy-6-formylsalicylic acid (III) is postulated to arise by nuclear hydroxylation¹³ of (II). Although this intermediate has yet to be detected, its structure is not unlike the 'orsellinic units' found in the lichen depsides¹⁴. Decarboxylation of (III) affords gentisaldehyde (VIII) directly, whereas oxidative decarboxylation would give rise to gentisic acid (IV). The mechanism of the conversion of (II) to the gentisic series by way of (III) is subject to experimental examination with isotopic carbon, since in each of the foregoing pathways a different carboxyl can be lost.

It is not yet clear whether gentisaldehyde or gentisic acid is closer to the open chain 'pre-patulin' (V or Va). From the replacement experiments it would appear that gentisic acid is more directly related to patulin (VI) than is the aldehyde, as might well be expected from the fact that the acid and patulin have the same empirical formula. Certainly, however, BIRKENSHAW's attractive hypothesis⁶ or some variation thereof seems at this point to have promise of explaining the actual mechanism of this interconversion.

The effects of trace metals in altering the ratios of metabolic products are probably exerted at the following points: either the two step oxidation of (I) to (II), blocking of which can result in the accumulation of 6-methylsalicylate in the medium, or at the oxidative *vs* straight decarboxylation of (III), which results in the primary accumulation of either gentisaldehyde or of gentisic acid. The accumulation of the last mentioned metabolites under low iron concentration may be related to the finding that the analogous homogentisic acid oxidase of animal tissues¹⁵ is an iron requiring enzyme. Reduction of gentisaldehyde by glycolytic fragments or enzymatic dismutation of this compound will produce gentisyl alcohol (IX), which was originally found as a metabolite in *P. patulum*¹⁶.

The second group of compounds, pyrogallol (XIII), *p*-hydroxybenzoic acid (XII), and anthranilic acid are more closely allied to the well characterized components

of the aromatic amino acid pathway from shikimic acid¹⁷. Accordingly, we hypothesize that pyrogallol may arise from shikimate via gallic acid (XI). The latter can be conceived of coming either by some direct aromatization of shikimic acid, or more likely from oxidation of *p*-hydroxybenzoate. Indeed, preliminary evidence by paper chromatographic and color tests indicated that gallate and shikimate can be found in the broth of strain 2159A, and the conversion of shikimic acid to *p*-hydroxybenzoate in replacements has already been mentioned.

In this connection, it must be pointed out that BIRCH *et al.*¹⁸, working with the related *P. griseofulvum*, conclude that 6-methylsalicylic acid arises from the direct 'head to tail' condensation of acetate. This is to a measure borne out by our experiments with acetate in replacement media. However the acetate hypothesis cannot be directly invoked to explain pyrogallol formation since condensation followed by cyclization of C₂ units results in meta orientated polyphenolic compounds. Nor does the conversion to pyrogallol from any of the products related to 6-methylsalicylic acid appear any the more likely. An outside alternative is that pyrogallol is formed from nuclear hydroxylation of resorcinol. The possibility of such ortho oxidations in nature has been considered by SESHADRI¹⁴ in a discussion of the C₈ lichen substances. A search for the presence of resorcinol or for meta hydroxylated acids in *P. patulum* has failed to uncover evidence for this type of substance.

We are faced therefore with the consideration that within the same microorganism two pathways toward aromatization might co-exist: the one from hexose to shikimic acid and thence to pyrogallol, *p*-hydroxybenzoic and the aromatic amino acids, and the other from hexose, possibly through acetate to 6-methylsalicylic acid and to patulin. If both pathways are present we might also expect to find that there are one or more points of convergence. We are planning to resolve some of these questions by means of mutant, isotopic tracer, and enzymological techniques.

Zusammenfassung

Ausser Patulin, Gentisinsäure und 6-Methylsalicylsäure wurden die folgenden Verbindungen als Metaboliten von *P. patulum* Stamm 2159A gefunden: 6-Formylsalicylsäure, 3-Oxyphthalsäure, Pyrogallol, *p*-Oxybenzoesäure und Anthranilsäure. Eine aliphatische Vorstufe von Patulin und eine Substanz vom Depsidtypus konnten noch nicht näher identifiziert werden. Die möglichen Stoffwechselzusammenhänge zwischen diesen Verbindungen wurden diskutiert.

¹⁷ B. D. DAVIS, *A Symposium on Amino Acid Metabolism* (Baltimore 1955), p. 799. – D. B. SPRINSON, *A Symposium on Amino Acid Metabolism* (Baltimore 1955), p. 817.

¹⁸ A. J. BIRCH, R. A. MASSEY-WESTROPP, and C. J. MOYE, *Austral. J. Chem.* 9, 539 (1955).

CONGRESSUS GREAT BRITAIN

Symposium on the Ecology of Soil Fungi

Liverpool, 19th to 21st of August 1958

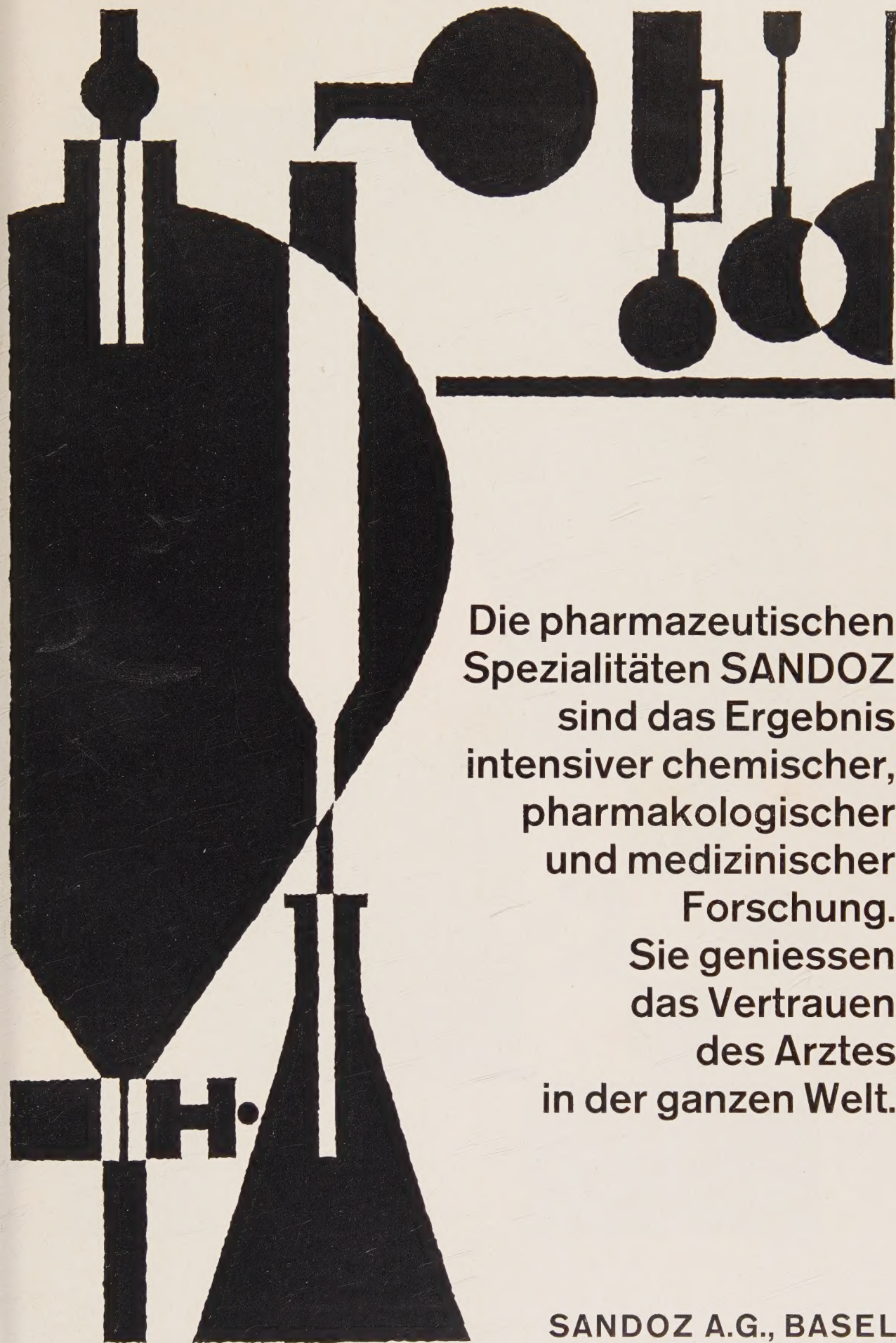
A symposium on the Ecology of Soil Fungi will be held in the Hartley Botanical Laboratories, The University, Liverpool, England, on the 19th to 21st of August 1958. Details of the symposium may be obtained from Dr. D. PARKINSON, The Hartley Botanical Laboratories, The University, Liverpool, England.

¹³ J. H. WEISBURGER, E. K. WEISBURGER, and H. P. MORRIS, *Science* 125, 503 (1957).

¹⁴ T. R. SESHADRI, *Exper. Suppl.* II, 258 (1955).

¹⁵ D. I. CRANDALL, *A Symposium on Amino Acid Metabolism* (Baltimore 1955), p. 867.

¹⁶ J. H. BIRKENSHAW, A. BRACKEN, S. A. MICHAEL, and H. RASTRICK, *Lancet* 245, 625 (1943). – A. BRACK, *Helv. chim. Acta* 30, 1 (1947).



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